

CREATINE AND CREATININE METABOLISM

By

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PREFACE

IT HAS now been almost 16 years since the appearance of A. Hunter's monograph on Creatine and Creatinine, which covered the literature up to and including 1926. It would seem that the time has arrived to pause and evaluate the increasing amount of experimental evidence that has been published in recent years in this important field. Many advances have been made. Especially is this true in regard to the origin of creatine and creatinine and the functions of creatine in the body.

Since it is impossible to discuss creatine and creatinine metabolism without considering its intimate relation to various other metabolic processes, I shall also attempt in this monograph to include the following topics: carbohydrate metabolism, muscular contraction, phosphate bond energy, phosphorylation and respiration, physical fitness, nutritional muscular dystrophy, the vitamins, hormones, etc., and creatine-creatinine metabolism in the myopathies and diseases of the heart.

It is obvious that this attempt will be a difficult one. Many of the older theories of creatine and creatinine metabolism are so well established in the minds of many investigators from the results of the earlier work listed in Hunter's monograph that any attempt to change or alter them has met with much opposition. It is certain, at the present time, that erroneous conclusions were drawn by many of the earlier workers. In no field of research do the experimental conditions employed have so much effect upon the results obtained as in this field.

Several reviews and monographs in the field have appeared since 1926. In the opinion of the writer, however, none of these gave the true status of the subject at the time that they were written. There is much duplication of the data given and conclusions reached by Hunter, and accepted theories of the earlier workers are considered to be established. This is seen especially in several clinical reviews of the subject.

Emphasis is placed here upon the results of the last 15 years' work in the field, with applications, wherever possible, to human metabolism and to diseases of the muscles and heart. Detailed instructions for the determination of creatine and creatinine in body tissues and fluids by the newer techniques are given in the hope that they may be used successfully by technicians not especially trained in analytical chemistry.

It was necessary to draw heavily upon the excellent reviews of Meyerhof, of Lipmann and of Kalckar in compiling the material in Chapter XIV. Grateful acknowledgment is made to my many colleagues who assisted in the work of our laboratory. Dr. Philip Pizzolato, of the Department of Pathology of Charity Hospital, has collaborated in our studies for the last 7 years. Without his help many of the results listed in the present monograph would not have appeared. I am also indebted to Professor Carl L. A. Schmidt and Dr. Fritz Lipmann for reviewing the manuscript and offering many constructive criticisms. My best thanks are due my secretary Miss Marion Blessey who typed the manuscript twice and to my wife for many valuable services in the preparation of the manuscript. Frances M. Troescher also gave much help in checking many small details.

New Orleans, La.

FOREWORD

It is a common custom among businessmen to take stock once a year. This is not only necessary in order to draw up an accurate balance sheet but it possesses the advantage that items which have become shop worn or are only seldom or no longer called for may be disposed of and be replaced by newer stock. In like manner it is highly desirable for the scientist now and then to gather all of the data in his field of endeavor, to sift them, to reappraise current and accepted views on the basis of both old and newer experimental data, to point the light of constructive criticism upon work hastily carried out and at times not well planned, and finally to weave the best into a pattern of thought. The conclusions may not be final. They may, however, serve as a stimulus for further work so that eventually the facts will come to light.

The subject of creatine and creatinine metabolism has indeed been a controversial one. The late Professor Lafayette B. Mendel once told the writer that it might be wise to forget all of the work that had been done in this field and to start anew. This was merely a way of expressing the confusion that existed at the time. Some of the pioneer work is as valid today as on the day it was published. This statement is not, however, to be taken as indicating that many more factors bearing on the subject have not been brought to light since that time.

The use of isotopes for the study of creatine and creatinine metabolism has pointed the way for a thorough and exhaustive restudy of the problem. Only a small beginning has thus

far been made. Eventually the source of all of the atoms in these molecules will have to be ascertained and in like manner the metabolic steps in which these atoms participate will have to be determined.

It is doubtful that in a system as complex as the animal body, which we may consider as a multiphase and multi-component system, reactions proceed along only one path. In the opinion of the writer, every component of the metabolic pool influences every other component to some degree, be it large or small. If we consider that under constant conditions the reactions in the body proceed at a steady state, is it not reasonable to expect that when an amount of a substance greater than participating in the steady state is fed or injected, the steady state will be altered?

Some of those who have worked on creatine and creatinine metabolism have only taken into account the products that have appeared in the urine; others have directed their attention chiefly to those compounds that can be determined in the tissues of the animal. Some conclusions have probably been drawn from experiments in which too few animals were employed and the period of study was all too short. It is the writer's opinion that it is always desirable and at times necessary to make simultaneous studies of the excretory products and the products that are contained in the tissues of the animal, to employ ample numbers of animals in order to minimize or at least to ascertain the extent of biological variability, and finally to carry out the experiments for a sufficient length of time so that some degree of steady state under the conditions of the experiment may be established. Even then the experimental data may apply only to the particular experimental conditions and not necessarily to the normal animal. It is indeed difficult at times to set up an experiment that may not alter or markedly influence normal processes. It is always desirable to simulate normal processes so far

as possible and to weigh the results with due consideration to deviations from the normal.

Many of our current views differ decidedly from those which were accepted only a comparatively short time ago. It is not unreasonable to assume that views will continue to change. This represents scientific progress. Hypotheses are only useful in that they provide a stimulus for further work. They will only survive and, to quote from Beaumont, "their worth will be best determined by the foundation on which they rest—the incontrovertible facts."

CARL L. A. SCHMIDT

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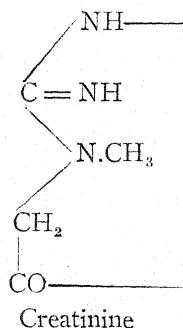
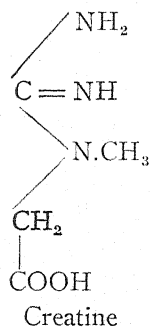
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CHAPTER I

INTRODUCTION

CREATINE was first isolated from meat extract by Chevreul in 1886. Since this time the origin and function of this interesting substance have baffled each generation of physiologists and biochemists. Creatine is methyl guanidine acetic acid and its internal anhydride is creatinine:



From 1886 until 1941 there have been four distinct periods of investigation. The first extends from 1886 until 1905. In this period the chemistry of creatine and creatinine and their occurrence and distribution in body tissues and organs was studied. The second period extends from 1905 until about 1927. This period opened with the late Professor Folin's (1, 2, 3) introduction of his classical method of utilizing the Jaffé reaction with alkaline picrate for the de-

termination of creatine and creatinine in body tissues and fluids. An immense amount of data, reviewed by Hunter (1) in 1927, dealt with the effect of sex, age, exercise, etc., on the distribution and excretion of these two bodies. The third period was opened in 1927 with the brilliant discovery of creatine phosphate by Fiske and Subbarow (1) and the Eggletons (2) and of its functions in muscular contraction and carbohydrate metabolism by Meyerhof, Lundsgaard, Lohmann and others. The fourth period also began about this time. Refined methods of analysis were introduced by Miller and Dubos by which the true creatinine and creatine contents of body tissues and fluids could be determined.

Another important contribution of this period was the discovery of the origin of creatine and creatinine from the proteins and amino acids of the diet and of the possible mechanism of the methylation of glycocyamine to creatine. Much new information on the significance of creatinuria in health and disease was determined. The fact that creatinine could be transformed into creatine, which has long been known but not accepted, and the rôle of water or saline in the process was discovered. The production of nutritional muscular dystrophy in animals by feeding upon unbalanced diets lacking vitamin E marked a brilliant advance in this field even though the practical applications of these discoveries in the human myopathies is not clear. Since it was definitely shown that glycine could increase muscle creatine, it was to be expected that this substance, as well as many others, would be introduced in the treatment of the human myopathies. It was also shown that glycine (and creatinine) ingestion serves to increase resistance to fatigue and that it increases the energy output in man.

Results during the last 15 years show that some of our previous theories of creatine and creatinine metabolism need revision. The newer concepts would be the following:

(1) Creatine and creatinine are not products of an endogenous metabolism as compared to an exogenous metabolism. No distinction is to be drawn between these two types of metabolism.

(2) Creatinine excretion is not necessarily dependent upon the body weight of the individual and the creatinine coefficient has little, if any, physiological significance.

(3) Administered creatine is not changed into creatinine in the tissues. On the other hand administered creatinine is transformed *in situ* into creatine and greatly stimulates the excretion of both creatine and creatinine. This leaves the biological relation between body creatine and creatinine open, since the metabolism of administered creatine and creatinine is different from body creatinine and creatine, *i.e.*, formed during the usual course of protein metabolism.

(4) Water, or saline, increases the formation of creatine from creatinine.

(5) The influence of some of the hormones, especially the posterior pituitary hormones and the adrenal cortical hormones, upon creatine formation from creatinine was due to their water and salt retaining effects.

(6) Creatinine excretion may be fairly constant in a given individual, but may differ widely in different individuals.

(7) There is no relation between basal or endogenous metabolism and creatinine excretion.

(8) The tissues contain a *creatine oxidase* and a *creatinine oxidase* which oxidize, or cause the disappearance of, creatine and creatinine, respectively. *Creatinine hydrazase* accelerates the transformation of creatinine into creatine. *Creatine anhydrazase* does not exist in the tissues.

(9) The creatine tolerance test is simply a creatine oxidation test and as such has little clinical significance.

(10) The creatinine clearance test of kidney function has lost much of its former physiological significance.

(11) Creatinuria is not necessarily due to a breakdown of glycogen (Brentano), but may be due to many unspecific causes (Wang, Thomsen). It is due chiefly to a lack of phosphate donors in the tissues for the formation of creatine phosphate (Beard), or to any disturbance in the metabolism of adenylypyrophosphate, creatine phosphate or carbohydrate disintegration products (Wang).

(12) The relation of creatine to glycolysis, phosphorylations, oxidations, reductions and respiration is becoming clearer.

(13) Creatine and creatinine are both products of protein metabolism in that they arise in the body from practically any of the amino acids. The amino group of the amino acid is necessary for creatine formation.

(14) The formation of glycocyamine occurs in the muscles from urea and glycine and in the kidney from arginine and glycine. Methylation occurs in the liver. Glycine, methionine, choline or betaine or other methylated amino compounds may furnish the methyl group necessary for this process.

(15) Substances furnishing this available methyl are also necessary in the diet to prevent the formation of fatty livers and degeneration of the kidneys.

(16) Muscular exercise does not usually affect the creatine-creatinine excretion.

(17) There is probably no relation between thiamin chloride, cocarboxylase, nicotinamide, and 2-methylnaphthoquinone and muscle creatine. Ingestion of thiamin chloride, does, however, augment the increased energy output due to glycine ingestion. On the other hand pyridoxin, nicotinic acid and vitamin E (α -tocopherol) gave increases from 20 to 36 per cent in muscle creatine. In some of these cases creatine excretion was also increased.

(18) Ingestion of glycine, as well as many other sub-

stances, may give increases in creatine excretion and some clinical improvement in the myopathies.

(19) In cardiac conditions, such as heart failure, there is a lowered creatine and phosphate content. The administration of glycine with thiamin chloride is indicated in this type of syndrome.

(20) Creatinuria as seen in the clinic may be due to a variety of conditions and is by no means specific for diseases of the muscles. Before its significance can be judged it is necessary to know the approximate protein and water intake of the patient. The administration of glycine should always be with a *diet rich in protein*, otherwise the glycine will be incorporated into the tissue proteins rather than into muscle creatine.

(21) The diagnostic value of the plasma creatinine determinations was indicated.

(22) Introduction of the specific creatinine enzymatic method for the determination of creatine and creatinine by Miller and Dubos has shown that it was "apparent creatine" rather than true creatine that had been determined with the Jaffé reaction in the past. It was observed that true creatinine comprises from 80 to 100 per cent of the chromogenic material of serum and plasma and from 30 to 50 per cent in the red cells. In urines from normal and nephritic individuals, true creatinine constituted nearly 100 per cent of the chromogenic material. Practically all of the chromogenic material in muscle filtrates is also true creatinine. However, in skeletal and heart muscle, testes and brain of the rat, the true creatine averages from 83 to 96 per cent of the apparent creatine, while, in intestinal muscle, pancreas, spleen, lung and liver, the true creatine amounts to only 16 to 65 per cent of the apparent value.

(23) The protein of muscle, myosin, is identical with adenylpyrophosphatase which liberates phosphate from

adenylpyrophosphate to supply energy for muscular contraction.

On the whole much progress has been made which will open up new avenues of approach for future experimental work especially in regard to the physiology of creatine and creatinine.

CHAPTER II

DETERMINATION OF CREATINE AND CREATININE IN BODY TISSUES AND FLUIDS

MANY INVESTIGATORS have proposed methods for the determination of creatine and creatinine in body tissues and fluids. Since most of these methods have never come into general use, no mention will be made of them here. The first accepted quantitative method for the determination of creatine and creatinine was the adaption of the Jaffé reaction with alkaline picrate, introduced by the late Professor Folin (4, 5) many years ago. This method, with the modifications to be given below, is generally considered to be the standard for the determination of these substances at the present time.

DETERMINATION OF PREFORMED CREATININE IN URINE

Reagents.

1. Saturated picric acid (the picric acid must be pure).
2. 10 per cent solution of sodium hydroxide.
3. Standard creatinine solution. (Dissolve 1,000 mg. of pure creatinine in 1,000 cc. of N/10 HCl. 1 cc. = 1 mg. creatinine.)

Procedure.

1. Collect an *accurate 24-hour specimen* of urine in a bottle containing 10 cc. of toluene, 10 cc. of N HCl. or

other suitable preservative. With a colorimeter carry out the determination as follows:

2. Pipette 1 (or 2 cc.) of the urine into a 100 cc. volumetric flask.
3. Pipette 1 cc. = 1 mg. of the (creatinine) standard into another 100 cc. volumetric flask.
4. Add 10 cc. of the saturated picric acid and 1.5 cc. of the 10 per cent NaOH to each flask and shake well.
5. Allow to stand 10 minutes and make up to the 100 cc. mark with distilled water and again shake thoroughly.
6. While the colors are developing, adjust the field in the colorimeter as follows: Add standard solution to each cup and adjust each plunger to the reading of 15 mm. Move the globe in the bottom of the instrument back and forth until exactly the same color is present on each side of the field. (IT IS MOST IMPORTANT THAT THIS OPERATION BE CARRIED OUT BEFORE EACH SERIES OF DETERMINATIONS; OTHERWISE ALL RESULTS WILL BE IN ERROR.) The same person who makes this adjustment should also read the colors developed in the determinations.
7. At the end of the 10 minute period pour out the standard solutions and rinse the cup on the left hand side with a little of the creatinine standard, and then fill the cup not more than $\frac{2}{3}$ full with the creatinine standard. Set the plunger at 15 mm. Keep this standard in place for all determinations.
8. Do likewise with the unknown urine sample in the right hand cup.
9. Rotate the right hand field up and down until the colors match.
10. Take reading of the standard (15) and of the unknown.

(It is very difficult for a beginner to match these colors accurately. However, with a little practice this can be done. Care must be taken to use the *same* pipette to measure accurately both the standard and all of the urine samples. If this is not done the error made will be multiplied by the total volume of the 24-hour urine sample and may be considerable. This is also important in the determination of the "total creatinine" below, since the creatine is the difference between the total and preformed creatinine.)

Calculation:

$$\frac{\text{Reading of the Standard} \times 1}{\text{Reading of the Unknown}} \times \frac{24 \text{ hour volume of urine in cc.}}{1 \text{ (or 2) cc.}} = \text{mg. of creatinine per day.}$$

Example. Suppose 1,500 cc. of urine were collected in 24 hours and 1 cc. of this was taken for analysis, then,

$$\frac{\text{Reading of the Standard (15)} \times 1}{\text{Reading of the Unknown (15)}} \times \frac{1,500}{1 \text{ (cc.)}} = 1.5 \text{ gms. creatinine per 24 hours.}$$

DETERMINATION OF TOTAL CREATININE IN URINE

1. Pipette 1 cc. of the 24 hour specimen of urine into a 100 cc. volumetric flask.
2. Add 1 cc. of (1 to 4) HCl and autoclave at 15 lbs. pressure for 30 minutes.
3. Cool and add 10 cc. saturated picric acid followed by 3 cc. of 10 per cent NaOH, shake and allow to stand 10 minutes.
4. Dilute to the mark with distilled water and compare in the colorimeter as described above for preformed creatinine.

Calculation:

$$\frac{\text{Reading of Standard} \times 1}{\text{Reading of the Unknown}} \times \frac{24 \text{ hour volume of urine in cc.}}{1 \text{ (cc.)}} = \text{mg. total creatinine per day.}$$

Example: If the same urine sample employed above for the determination of preformed creatinine was used the calculation would be

$$\frac{\text{Reading of Standard (15)} \times 1}{\text{Reading of the Unknown (13)}} \times \frac{1,500}{1 \text{ cc.}} = 1.731 \text{ gms. total creatinine per day.}$$

Now the preformed creatinine was 1.500 gm. per day. Hence the difference between the total and preformed creatinine is expressed as "creatine as creatinine." This difference when multiplied by 1.16 is creatine.

Example: T.C. — P.C. = 1.731 — 1.500 = 0.231 gm. creatine as creatinine per day.

$$0.231 \times 1.16 = 0.268 \text{ gm. creatine per day.}$$

This latter calculation is usually not made in practice because the simple calculation can easily be made if anyone should wish to know the creatine content of the urine. Creatine is, therefore, usually expressed "creatine as creatinine" in the literature.

The introduction of photoelectrical instruments has greatly refined the determinations of creatine and creatinine in body tissues or fluids. The instrument to be used must be calibrated against pure creatinine in alkaline picrate and a concentration curve drawn. The author has used the Fisher electrophotometer (Fisher Scientific Company, Pittsburgh, Pa.) for a number of years with highly satisfactory results. After calibration of this instrument, the values given in Table 1 were obtained from the reading of the large dial on the instrument.

ADJUSTMENT OF THE INSTRUMENT

Before the determination is carried out the instrument must be accurately adjusted. This is done as follows:

1. Place the gelatin "B Filter No. 58" (Eastman Kodak Company, Rochester, N. Y.) in place and fill the cup with distilled water. (Always fill the cup with distilled water when not in use and keep the gelatin filter in the ice box.)
2. Turn the "Range Switch" to position 1 for the determination of creatinine and let it stay there.
3. Turn on the current to the instrument and galvanometer and throw the switch of the instrument to the "On" position. The needle on the voltmeter will turn to the right in the neighborhood of the red line just beyond position 5. The light image on the galvanometer will be near the "0" position. Allow the instrument to warm up for about 10 minutes.
4. At the end of this time adjust the needle on the voltmeter to coincide with the red line just beyond the position 5. *The needle must stay here during the determinations.* If it should deviate back to about 4.5, the determinations must be repeated after the storage battery has been charged. (The needle on the voltmeter may be adjusted to the correct position by gently turning the "Voltage" switch on the instrument to the right.)
5. Turn the large dial on the instrument to the "0" position.
6. Then adjust the midline of the light image on the galvanometer to the "0" position. (This is done by adjusting the switches "Initial Null Coarse" for a large deviation and "Initial Null Fine" for a small deviation. A little practice will be required to do this.)
7. Before the instrument is used be sure that the light image on the galvanometer is at the "0" position, the large dial at the "0" position on the instrument, and that the pointer on the voltmeter is on the red line just beyond position 5.

Procedure. This is the same as described for the determination of preformed creatinine above.

1. Pipette 1 cc. of the creatinine standard ($= 1$ mg. creatinine) into a 100 cc. volumetric flask and add 10 cc. of saturated picric acid and 1.5 cc. of 10 per cent NaOH. Shake well. At the end of 10 minutes dilute to the mark with distilled water and shake. Fill the cup almost full with this standard. Dry off both sides of the cup and place in its proper place in the instrument. Close the window and turn the large dial to position 38 and adjust the light image on the galvanometer to the "0" position as before. This means that a scale reading of 38 is equivalent to 1 mg. of creatinine per 100 cc. of the standard. (*This standardization of the instrument must always be carried out before and after each set of determinations.*) A reading of 38 for the standard will usually be observed after all samples are run.
2. Empty the cup and turn the large dial back to the "0" position on the instrument.
3. In like manner pour the unknown urine sample, which was prepared at the same time as the creatinine standard, into the cup and close the window. The light image on the galvanometer will deviate to the right of the "0" position. Bring it back exactly to this position by turning the large dial to the left.
4. Take the reading on the large dial and convert it to creatinine by reference to the values in Table I.
5. After all determinations are completed, fill up the cup with distilled water and place the gelatin filter in the ice box.

Calculation: Suppose 1 cc. of a 24-hour specimen of urine (1,500 cc.) was used in the determination. A reading of 38 was obtained, which is equivalent to 1 mg. of creatinine in the sample or 1.5 gms. for the 24-hour specimen.

TABLE 1

MILLIGRAMS CREATININE BY ELECTROPHOTOMETER

R.	Mg.	R.	Mg.	R.	Mg.	R.	Mg.	R.	Mg.	R.	Mg.
1.0	0.010	5.8	0.058	10.6	0.110	15.4	0.235	20.2	0.355	25.0	0.500
1.2	0.012	6.0	0.060	10.8	0.120	15.6	0.240	20.4	0.360	25.2	0.506
1.4	0.014	6.2	0.062	11.0	0.125	15.8	0.245	20.6	0.365	25.4	0.512
1.6	0.016	6.4	0.064	11.2	0.130	16.0	0.250	20.8	0.370	25.6	0.518
1.8	0.018	6.6	0.066	11.4	0.135	16.2	0.255	21.0	0.375	25.8	0.524
2.0	0.020	6.8	0.068	11.6	0.140	16.4	0.260	21.2	0.382	26.0	0.530
2.2	0.022	7.0	0.070	11.8	0.145	16.6	0.265	21.4	0.389	26.2	0.536
2.4	0.024	7.2	0.072	12.0	0.150	16.8	0.270	21.6	0.396	26.4	0.542
2.6	0.026	7.4	0.074	12.2	0.155	17.0	0.275	21.8	0.403	26.6	0.548
2.8	0.028	7.6	0.076	12.4	0.160	17.2	0.280	22.0	0.410	26.8	0.554
3.0	0.030	7.8	0.078	12.6	0.165	17.4	0.285	22.2	0.418	27.0	0.560
3.2	0.032	8.0	0.080	12.8	0.170	17.6	0.290	22.4	0.426	27.2	0.568
3.4	0.034	8.2	0.082	13.0	0.175	17.8	0.295	22.6	0.434	27.4	0.576
3.6	0.036	8.4	0.084	13.2	0.180	18.0	0.300	22.8	0.442	27.6	0.584
3.8	0.038	8.6	0.086	13.4	0.185	18.2	0.305	23.0	0.450	27.8	0.590
4.0	0.040	8.8	0.088	13.6	0.190	18.4	0.310	23.2	0.455	28.0	0.600
4.2	0.042	9.0	0.090	13.8	0.195	18.6	0.315	23.4	0.460	28.2	0.606
4.4	0.044	9.2	0.092	14.0	0.200	18.8	0.320	23.6	0.465	28.4	0.612
4.6	0.046	9.4	0.094	14.2	0.205	19.0	0.325	23.8	0.470	28.6	0.618
4.8	0.048	9.6	0.096	14.4	0.210	19.2	0.330	24.0	0.475	28.8	0.624
5.0	0.050	9.8	0.098	14.6	0.215	19.4	0.335	24.2	0.480	29.0	0.630
5.2	0.052	10.0	0.100	14.8	0.220	19.6	0.340	24.4	0.485	29.2	0.636
5.4	0.054	10.2	0.105	15.0	0.225	19.8	0.345	24.6	0.490	29.4	0.642
5.6	0.056	10.4	0.110	15.2	0.230	20.0	0.350	24.8	0.495	29.6	0.648
29.8	0.654	35.0	0.850	40.2	1.11	45.4	1.43	50.6	1.86	55.8	2.47
30.0	0.660	35.2	0.860	40.4	1.12	45.6	1.44	50.8	1.88	56.0	2.50
30.2	0.668	35.4	0.870	40.6	1.13	45.8	1.45	51.0	1.90	56.2	2.53
30.4	0.676	35.6	0.880	40.8	1.14	46.0	1.46	51.2	1.92	56.4	2.56
30.6	0.684	35.8	0.890	41.0	1.15	46.2	1.47	51.4	1.94	56.6	2.59
30.8	0.692	36.0	0.900	41.2	1.16	46.4	1.48	51.6	1.96	56.8	2.62
31.0	0.700	36.2	0.910	41.4	1.17	46.6	1.49	51.8	1.98	57.0	2.65
31.2	0.706	36.4	0.920	41.6	1.18	46.8	1.50	52.0	2.00	57.2	2.68
31.4	0.712	36.6	0.930	41.8	1.19	47.0	1.51	52.2	2.02	57.4	2.71
31.6	0.718	36.8	0.940	42.0	1.20	47.2	1.54	52.4	2.04	57.6	2.74
31.8	0.724	37.0	0.950	42.2	1.21	47.4	1.57	52.6	2.06	57.8	2.77
32.0	0.730	37.2	0.960	42.4	1.22	47.6	1.60	52.8	2.08	58.0	2.80
32.2	0.736	37.4	0.970	42.6	1.23	47.8	1.63	53.0	2.10	58.2	2.83
32.4	0.742	37.6	0.980	42.8	1.24	48.0	1.66	53.2	2.12	58.4	2.86
32.6	0.748	37.8	0.990	43.0	1.25	48.2	1.67	53.4	2.14	58.6	2.89
32.8	0.754	38.0	1.00	43.2	1.27	48.4	1.67	53.6	2.16	58.8	2.92
33.0	0.760	38.2	1.01	43.4	1.28	48.6	1.68	53.8	2.18	59.0	2.95

TABLE 1, *cont'd*

MILLIGRAMS CREATININE BY ELECTROPHOTOMETER

<i>R.</i>	<i>Mg.</i>	<i>R.</i>	<i>Mg.</i>	<i>R.</i>	<i>Mg.</i>	<i>R.</i>	<i>Mg.</i>	<i>R.</i>	<i>Mg.</i>	<i>R.</i>	<i>Mg.</i>
33.2	0.768	38.4	1.02	43.6	1.29	48.8	1.69	54.0	2.20	59.2	2.98
33.4	0.776	38.6	1.03	43.8	1.31	49.0	1.70	54.2	2.23	59.4	3.01
33.6	0.784	38.8	1.04	44.0	1.33	49.2	1.72	54.4	2.26	59.6	3.04
33.8	0.792	39.0	1.05	44.2	1.34	49.4	1.74	54.6	2.29	59.8	3.07
34.0	0.800	39.2	1.06	44.4	1.36	49.6	1.76	54.8	2.32	60.0	3.10
34.2	0.810	39.4	1.07	44.6	1.37	49.8	1.78	55.0	2.35		
34.4	0.820	39.6	1.08	44.8	1.38	50.0	1.80	55.2	2.38		
34.6	0.830	39.8	1.09	45.0	1.40	50.2	1.82	55.4	2.41		
34.8	0.840	40.0	1.10	45.2	1.41	50.4	1.84	55.6	2.44		

It is best to have the urine sample so dilute that the reading will not go much beyond 38. In the case of human urines use 0.5 cc. of the 24-hour specimen, making the corresponding changes in the calculations, or dilute the specimen with an equal volume of water and use 1 cc. as usual.

DETERMINATION OF CREATINE IN URINE

In this determination the total creatinine (creatinine + creatine) is determined in one sample of urine and the preformed creatinine in another. The difference between these two values $\times 1.16$ is creatine. Creatinine is the anhydride of creatine and by heating the sample with HCl any creatine present will split off water to form creatinine, which is stable in acid solution.

Procedure.

1. Use three 100 cc. volumetric flasks, *a*, *b*, and *c*, as follows:

Into *a* pipette 1 cc. of the creatinine standard.

Into *b* pipette 1 cc. of the urine (for preformed creatinine).

Into *c* pipette 1 cc. of the urine and add 1 cc. of (1 to 4) HCl (for total creatinine).

2. Set *a* and *b* aside.
3. Place *c* in the autoclave and heat at 15 lbs. pressure for $\frac{1}{2}$ hour. Cool to room temperature.
4. Add 10 cc. of picric acid and 1.5 cc. of 10 per cent NaOH to *a* and *b* and shake.
5. Add 10 cc. of picric acid and 3 cc. of 10 per cent NaOH to *c* and shake.
6. Allow these three flasks to stand 10 minutes and then dilute each to the mark with distilled water.
7. Standardize the instrument as described above with *a* before step 6 is carried out.
8. Then determine the depth of color in *b* and *c* as described above.
9. $c - b = \text{creatinine}$, expressed as creatinine. For creatine, multiply the difference between *c* and *b* by 1.16.

DETERMINATION OF CREATINE IN TISSUES

Since there is very little preformed creatinine in tissues, the total creatinine in these tissues is determined and this is taken to represent their creatine content. The method of Rose, Helmer and Chanutin (2) has been used by the author for a number of years and gives excellent results.

Reagents.

1. 2 N sulfuric acid.
2. 2 N sodium hydroxide.
3. 10 per cent sodium tungstate.
4. 10 per cent sodium hydroxide.
5. Saturated (purified) picric acid.
6. Creatinine standard (1 cc. = 1 mg. of creatinine).

Procedure (for electrophotometer).

1. Kill the animal by a blow on the back of the head.
2. Remove about 1 gm. of muscle tissue from the hind leg as rapidly as possible and cut up fine with scissors.

3. Weigh this sample accurately in a previously weighed 50 cc. glass stoppered Erlenmeyer flask.
4. Add 20 cc. of 2 N sulfuric acid.
5. Cover flask with small beaker and autoclave for 45 minutes at 15 lbs. pressure. Cool to room temperature.
6. Transfer contents of the flask to a 100 cc. volumetric flask with about 20 to 30 cc. of distilled water.
7. Add 18 cc. of 2 N sodium hydroxide into the flask followed by 5 cc. of 10 per cent sodium tungstate.
8. Dilute to 100 cc. with distilled water, shake, allow to stand 5 minutes and filter.
9. Prepare the standard containing 1 mg. of creatinine per cc. in another 100 cc. flask as usual and standardize the electrophotometer.
10. Pipette 10 cc. of the clear filtrate from 8 into another 100 cc. flask.
11. Add 10 cc. of saturated picric acid and 3 cc. of 10 per cent NaOH to the flask (procedure 10). Let stand 10 minutes, dilute to the 100 cc. mark with distilled water, shake, and determine the color intensity and corresponding creatinine content from Table I as usual.

Calculation. Suppose that 1.276 gms. of muscle tissue were used in the analysis. The reading on the instrument was 29 (= to 0.63 mg. creatinine).

Then

$$\frac{0.63 \times 10}{1276} \times 100 = 0.49 \text{ per cent creatine (as total creatinine).}$$

The value of the reading (0.63 mg. creatinine) is multiplied by 10, since only 10 cc. of the 100 cc. (from step 10 representing the sample) were used for color matching, etc.

If the usual eye colorimeter is to be employed, use the

original procedure described by Rose, Helmer and Chanutin (2).

DETERMINATION OF CREATINE AND CREATININE CONTENT OF BLOOD

If the determination of the creatine and creatinine content of the blood is desired, use the procedures described in laboratory manuals of biochemistry. These determinations are chiefly of value in diagnosis of disease and are seldom required in research work (except in determinations of the creatinine clearance test of kidney function) and will be omitted here. The principle of the determinations is the same as described above after preparing a tungstic acid filtrate of the blood. In the analysis of muscle tissue and urine for creatine and creatinine, the use of the Miller, *et al.* (1, 2, 3, 4, 5) specific enzymatic methods are unnecessary since about 95 per cent of the chromogenic substances in muscle filtrates and urine is true creatinine (see next chapter). If these methods are used, the reader should consult the original publications.

The principle of this enzymatic method is as follows:

1. Determine the total and preformed creatinine of the 1 cc. sample of urine as described above, or use 10 cc. of the muscle filtrate. Record these values.

2. Pipette 1 cc. of the original urine (or 10 cc. of the neutralized muscle filtrate) into a 50 cc. Erlenmeyer flask. Add 5 cc. of the phosphate buffer of pH 7 together with a sufficient amount of the NC suspensions of the organisms, and place the unstoppered flask in the incubator at 37° for 30 minutes to 1 hour. Cool and filter if necessary.

3. Transfer the filtrate to a 100 cc. volumetric flask and add 10 cc. of picric acid and 1.5 cc. of 10 per cent NaOH and shake. Allow to stand 10 minutes, dilute to the mark with distilled water and determine the depth of the color in

the electrophotometer or with the colorimeter. If creatinine alone was responsible for the red color of the Jaffé reaction with alkaline picrate then this color will *not* develop in the sample used. The organisms oxidize the creatinine to other compounds which do not give the Jaffé reaction. However, some of the color of the Jaffé reaction may persist showing the presence of another closely related compound to creatine or creatinine or the presence of a substance, such as glucose, which reduces sodium picrate to sodium picramate, and since the latter solution would also be red, it would require the enzymatic method to distinguish this color from the red color produced by creatinine when added to alkaline picrate, or the Jaffé reaction.

The reading on the electrophotometer will give the concentration of this compound (as glycocyamidine, glucose, acetoacetic acid, etc.) and the value of this reading in terms of creatinine can be subtracted from the readings obtained under 1 above to get the amount of "true creatine or creatinine" and the amount of the "apparent creatine or creatinine" which would not be due to these substances at all. For instance only 16 per cent of the total creatinine of liver tissue is true creatinine as determined with the enzyme method, and the rest is "apparent creatinine," whose true nature is unknown at the present time.

4. Pipette 1 cc. of a solution of creatinine (= 1 mg.) or neutralize 1 cc. of the Creatinine-HCl standard in water with dilute NaOH, add the 5 cc. of phosphate buffer, and the same amount of the NC suspension as used above and incubate at 37° C. for ½ hour. Upon addition of the alkaline picrate to the flask NO color should develop. If the Jaffé reaction does develop the organisms are not active and a new suspension should be used.

• The flasks must be left *open* in the incubator during the reaction, the solution must be neutral, and the NC organisms

must destroy a sample of pure creatinine to show that they are active. The reader should consult the original publications for the growth and cultivation of these bacterial suspensions.

CHAPTER III

DISTRIBUTION OF TOTAL, TRUE AND APPARENT CREATINE IN THE BODY

THE TOTAL creatinine of the body tissues and fluids, *i.e.*, the total amount of chromogen that reacts with alkaline picrate to give the Jaffé reaction, is determined, as stated in the last chapter, by heating the urine with 1 to 4 HCl, and, after neutralizing the acid with NaOH, determining the total color developed after adding alkaline picrate. In this determination the creatine + creatinine (and other chromogenic substances, if present) are determined. It is, therefore, often necessary to know the amount of these three constituents present in the body tissues and fluids at any given time. The recent methods introduced by Miller and Dubos and their colleagues, referred to in the last chapter, has afforded a way of doing this.

In many cases the terms creatine and creatinine signified the "apparent" creatine and creatinine, but the adjective was hardly ever used in this connection. Since creatinine occurs only to a very slight extent in body tissues and fluids except the blood and urine, only creatine and its different forms will be considered here.

The lack of specificity of the alkaline picrate reaction for creatinine has been criticized ever since it was introduced by Jaffé in 1886. Although it is true that many compounds

give this reaction *in vitro*, it is also true that practically none of these compounds are present in body tissues and fluids. It has been supposed for many years that there was a chromogenic substance present in the blood which was not creatinine. In 1927 Gaebler and Keltch (1) stated that both normal and retention blood samples contained chromogenic substances other than creatinine and the same view was also emphasized by Bohn and Hahn (1). Matsumoto (1) boiled pigs' brain with absolute alcohol and extracted the residue with ether and finally with water. Most of the creatine was found in the alcoholic and water extracts, while the ether extract contained about 14 per cent of the total creatinine of the brain. Since creatine itself is insoluble in ether it was concluded that the previously reported values for brain creatine were too high. Until 1937, therefore, the determinations of creatine and creatinine in body tissues and fluids, listed in Hunter's Monograph, represented the "apparent" creatine and creatinine.

The introduction of the specific enzymatic methods for the determination of creatinine by Miller, Baker and Dubos (1, 2, 3, 4, 5), referred to in the last chapter, has marked a new era in the study of the distribution of these substances in body tissues and fluids. Determinations were made of the true creatinine content of the plasma, serum and red cells, and urine of normal individuals and of those suffering from various degrees of diminished kidney function. It was found that true creatinine comprises from 80 to 100 per cent of the chromogenic material of serum and plasma and from 30 to 50 per cent in the red cells. In urines from normal and nephritic individuals, true creatinine constituted nearly 100 per cent of the chromogenic material.

Baker and Miller (1) showed that practically all of the chromogenic material in muscle filtrates was true creatine. However, in skeletal and heart muscle, testes and brain of

the rat, the true creatine averages from 83 to 96 per cent of the apparent creatine, while, in the intestinal muscle, pancreas, spleen, lung and liver, the true creatine amounts to only 16 to 65 per cent of the apparent value. It was also found that the concentration of creatinine was highest in muscle, testes and brain, and considerably lower in kidney, pancreas, lung and liver. The concentrations of creatine and creatinine were roughly proportional in the different tissues, indicating an interrelationship of these two compounds in metabolism. The interesting observation was made that the concentration of creatinine in the spleen, lung, liver and pancreas was much lower than in an ultrafiltrate of plasma and this indicated that these tissues are concerned with the transformation of creatinine into creatine or other compounds. (Table 2.)

A possible criticism should be offered against the findings of Miller and Baker in this connection. It is of course realized that their work is very accurate and laborious, but this fact does not necessarily justify the finality of their conclusions. Usually only 2 rats were analyzed, together with a dog and a monkey. It is well known that the concentration of creatine in the muscles of different species of animals varies widely and Miller and Baker correctly stated that diet may account for the differences in concentration of creatine found by themselves and those of Chanutin (1). While their data are highly suggestive, nevertheless, more analyses should be conducted under definitely controlled dietary conditions to determine the magnitude of variations in the creatine content of different tissues.

It should be remembered that the bacteria used by Miller and coworkers produce the so-called "adaptive" enzymes on any media containing carbon and nitrogen, or other carbon compounds such as glucose, in the presence of ammonium carbonate. It is therefore necessary to grow the cultures on

TABLE 2

DISTRIBUTION OF CREATINE IN ANIMAL TISSUES
(mg./100 gm.)
(After Baker and Miller (1))

Tissue	RAT			DOG			MONKEY		
	Apparent Creatine	Residual Creatine	$\frac{\text{True}}{\text{Apparent}} \times 100$ Cent	Apparent Creatine	Residual Creatine	$\frac{\text{True}}{\text{Apparent}} \times 100$ Cent	Apparent Creatine	Residual Creatine	$\frac{\text{True}}{\text{Apparent}} \times 100$ Cent
Gastrocnemius	562	22	540	332	23	309	485	10	475
Diaphragm	459	31	428	398	23	375	446	15	431
Heart									
Muscle	217	21	196	344	17	326	217	18	199
Testis	324	22	302						
Brain	156	26	130						
Intestinal				150	21	129			92
Muscle	103	36	67						
Ovary	86	31	55	36	25	11	40	20	20
Spleen	57	42	15	47	20	27	50	31	19
Liver	37	31	6	54	48	6	62	22	38
Lung	47	33	14	31	22	9	31	23	40
Pancreas	74	32	42	34	24	10	49	24	8
Kidney	54	28	26	33	24	9	53	20	26
Pituitary				81	42	39			51
Submaxillary									62
Adrenal				44	34	10			
Uterus				39	32	7	36	4	32
									89

* $100 \times (\text{true creatine})/(\text{apparent creatine})$.

† Bürger (1) calculated that 98 per cent of the creatine in the human body was contained in the muscles. No data exist to disprove this statement. However, the data on distribution of true creatine in the rat and dog listed in the above table do not support Bürger's calculation.

creatinine if the suspensions obtained are to be used in the determination of creatine and creatinine. The interesting finding was made by Miller and Baker (5) that these suspensions will also oxidize sarcosine (methyl glycine). This fact suggests that sarcosine is an intermediate in the oxidation of creatinine.

Dubos (1) reviewed the literature on the adaptive production of enzymes by these bacteria. The specificity of the creatinine oxidases has been determined by testing them against a number of substrates related to creatinine. The addition of a methyl or acetyl group to the creatinine molecule inhibited the action of the enzymes while the removal of the methyl group in position 3 leaving glycoyamidine or its shift from position 3 to position 5 considerably retards the action of one of the enzymes and completely inhibits the action of the other. Dubos and Miller (2) showed that the enzyme which converts creatine to creatinine shows a similar specificity. This adaptive anhydrase offers an interesting example of the enzymatic combination of an amino and carboxyl group to form the —CONH— linkage, and this reaction may be useful in studying the metabolism of creatine and creatinine. It is also interesting that this enzyme was formed equally well when creatinine instead of creatine was added to the medium.

The nature of the "residual chromogen" (that part of the Jaffé reaction with alkaline picrate which is not destroyed by the creatinine enzymes of Miller and Dubos) has not been determined. Gerard and Tupikova (1) showed that in the resting oxygenated tissue the total creatine was phosphocreatine, free creatine and residual chromogen respectively in per cent as follows: nerve, 40, 40, 20; muscle, 50, 44, 4; and brain, 20, 60, 20. They stated that the residual chromogen was not creatine and that it may be bound to protein.

Some investigators in the past have denied the existence

of creatinine in the blood. The final answer to this problem is furnished by the studies of Miller and Dubos (2) who showed that from 80 to 100 per cent of the chromogenic material of the serum and plasma is *true creatinine*. Plasma from uremic patients, however, contained a large amount of non-chromogenic material which, in several patients, was proportional to the severity of the uremic toxemia.

In spite of the wide interest shown in the rôle of creatine and creatine phosphate in muscle metabolism in recent years, very few analyses of human voluntary muscle have been made. Corsaro, Mangun, and Myers (2) made comparisons of the creatine content of three separate muscles in 74 human autopsy cases. The average results were as follows: rectus abdominis, 405 mg.; psoas major, 402 mg.; sternocleidomastoid, 388 mg. per 100 gm. Their results are listed in Tables 3 and 4.

TABLE 3

CREATINE CONTENT OF HUMAN VOLUNTARY MUSCLE
(After Corsaro, Mangun and Myers (2))
Results are expressed in mg./100 gm.

No. of Cases	Muscle	CREATINE			Condition of Subject
		Aver- age	Maxi- mum	Mini- mum	
11	Pectoralis major	443	462	422	Normal
51	" "	405	538	212	Pathological
60	" "	395	564	282	"
74	Rectus abdominis	405	576	133	"
74	Psoas major	402	614	169	"
74	Sternocleidomastoid ...	388	696	169	"

From Table 3 it is seen that the creatine content of the psoas major, rectus abdominis, and pectoralis major are very nearly the same, while that of the sternocleidomastoid is slightly less. The values listed in Table 4 show that high values were found in uremia with creatinine retention, broncho-pneumonia and tuberculosis, while lower values were

TABLE 4
SUMMARY OF FINDINGS IN DIFFERENT CONDITIONS
(After Corsaro, Mangun and Myers (2))
Results are expressed in mg./100 gm.

No. of Cases	RECTUS ABDOMINIS			P SOAS MAJOR			STERNOCLEIDOMASTOID			Diagnosis
	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	
8	423	559	316	429	544	300	467	552	253	Uremia with creatinine retention
19	425	544	299	415	426	291	388	595	207	Broncho-pneumonia
9	443	576	236	432	525	301	426	696	216	Tuberculosis
6	389	490	284	385	559	253	365	495	306	Cancer
9	323	421	133	309	389	159	334	451	226	Acute inflammation
13	412	466	332	396	508	250	389	635	196	Circulatory involvement
4	347	381	316	372	450	300	306	354	253	Uremia and heart failure
6	415	504	333	458	614	378	391	480	169	Miscellaneous

found in the other conditions. In regard to these pathological findings it was stated that many factors affecting the general health of the individual may be reflected in the concentration of muscle creatine. The creatine content is lowered in the muscles when their nutritional condition is adversely affected.

Boyland (1) showed that human tumors contained creatine. There was no creatinine in rat tumors, but the creatine amounted to 30-60 mg. per 100 gm. of tissue. From one fourth to one third of the creatine is bound to phosphate and this creatine phosphate breaks down on standing. There was four times as much acid insoluble phosphorus as there is in skeletal muscle. Tumors also contain as much adenylyl pyrophosphate as does skeletal muscle. Lustig and Wachtel (1) stated that glycocyamine and creatine dissolved cancer cells *in vitro* and that guanidine derivatives prolonged the life of tumor bearing animals. These unusual results would be of great interest if confirmed.

At the present time with the introduction of photoelectric and spectrophotometric methods for measuring color intensities and with the use of the specific creatinine enzymes of Miller and Dubos, the student of creatine and creatinine metabolism can have a much higher degree of confidence in his results than heretofore, and criticisms of the lack of specificity of the Jaffé reaction for creatinine in body tissues and fluids are no longer justified. The writer predicts that, with the modifications discussed in this chapter, this reaction as introduced by Professor Folin in 1905 will remain the standard method for the determination of creatine and creatinine for many years to come.

CHAPTER IV

CREATINE-CREATININE CONTENT OF THE BLOOD. DIAGNOSTIC VALUE OF PLASMA CREATININE DETERMINATION

THE GREATER part of creatine is contained in the corpuscles, while creatinine is about equally distributed between cells and plasma. The creatine content of the blood is about 3 mg. per 100 cc. It is little changed in disease and has little diagnostic significance. It may be increased in cases of nitrogen retention and in the myopathies. Creatine phosphate does not usually occur in blood, but does so after the administration of creatine or creatinine (Abdon and Giesselson (2)). Blood creatinine is usually very constant and will average about 1 to 1.5 mg. per 100 cc. It is also increased in cases of nitrogen retention and a creatinine content of over 5 mg. per 100 cc. is considered of grave significance to the patient. Bohn and Hahn (1) showed that blood creatine is lowered in cases of hypertension. The studies of Miller, *et al.* (1, 2, 3, 4, 5), referred to in the last two chapters, shows that not all of the chromogenic material of serum, plasma and red cells is true creatinine. Therefore, if the true concentration of creatine and creatinine in the blood is desired, the enzymatic methods of Miller and Dubos should be used.

A few cases are on record where creatinine values con-

siderably higher than 5 mg. per 100 cc. have been reported with recovery (Watnabe (1), Bowman and Wolpaw (1) and Simpson (1)). Simpson's patient suffered from an automobile accident, followed by anuria, and his creatinine level reached 20 mg. per 100 cc. This type of case is, however, extremely rare and the high blood creatinine encountered in diseases of the kidney is to be looked upon as a serious prognostic sign. Both creatine and creatinine retention may occur in urological disorders and especially in the terminal stages of nephritis where the urea clearance falls below 20 per cent of the normal. McCord (1) stated that an analysis of blood creatinine values in 219 white and 298 negro subjects with renal diseases, at the New Orleans Charity Hospital, showed that higher levels are likely to occur in the negro. No explanation was given of this difference.

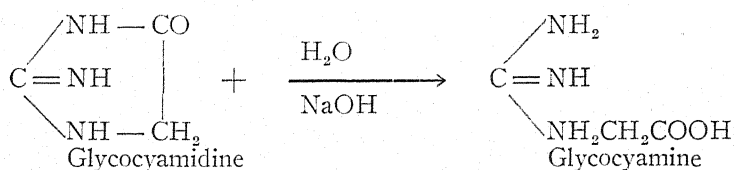
Cornil, *et al.* (1) showed that the average content of blood in creatine and creatinine was 15 mg. of each per liter. Higher values are pathological. The ratio creatinine (creatinine + creatinine) is normally 0.5, but is lower in certain diseases. Both substances increase proportionally in anuria concomitantly with urea. Creatine is increased in the blood in hepato-nephritis, in the terminal stage of cirrhosis, after operations and fever, during treatment with neoarsphenamine, in severe fatigue and after hyperthermia. Creatinemia with normal creatinine values is observed in the progressive myopathies, in Parkinsonianism and in pituitary cachexia. Increases in blood creatine are also generally associated with disturbed muscle function.

The elevation of the blood creatinine, except in rare instances, is of grave significance to the patient. It rises in the blood only when renal function becomes markedly impaired (Myers and Lough (5)). According to Myers and Muntwyler (6), who have reviewed this subject, several workers have stated or implied that the retention of creatinine went

hand in hand with urea, and therefore the prognostic value of an elevated blood creatinine was no greater than that of urea or non-protein nitrogen. Bruger and Mosenthal (1) stated, in this connection, "Our results show that normal creatinine values may be found in the blood with as much as 85 per cent of kidney function lost, as measured by the urea clearance test. When the urea clearance has fallen to about 5 per cent of normal and uremia is impending, the creatinine begins to mount in the blood." It would seem, therefore, that the prognostic value of a high blood creatinine, especially in diseases of the kidney, has lost none of its importance.

Major, *et al.* (6) showed that the compound they had previously designated as "guanidine" in the blood is probably glycoyamidine. In experimental renal insufficiency both with and without accompanying hypertension, there is an increase in "guanidine". In an attempt to isolate the "guanidine" Major, *et al.* employed tungstic acid filtrates from blood of dogs in the last stages of uranium nephritis. These filtrates were extracted with Lloyd's reagent and an eluate was prepared from it by means of a saturated solution of barium hydroxide. The "guanidine" was precipitated from these eluates by picric or phosphotungstic acids. The similarity of the color given by these more concentrated solutions with the ferricyanide-nitroprusside reagent to that given by a solution of glycoyamidine was striking. It was difficult to compare this color in the colorimeter with any of the guanidine derivatives such as arginine, glycoyamidine or creatinine except glycoyamidine. Most of the color attributed to "guanidine" in dog blood was shown to be due to arginine. But this substance in the blood of dogs suffering from uranium nephritis was not arginine. The Sakaguchi reaction was not increased under these conditions. Complete separation of the "guanidine" from creatinine was not possible in their studies. But if this substance in blood in renal insufficiency is made al-

kaline and allowed to stand there is a gradual increase in the Sakaguchi reaction. This increase in color parallels that of a solution of glycoyamidine treated in similar manner to the blood sample. These observations indicate that the substance is a guanidine derivative of an anhydric type and that under alkaline treatment the guanidine ring opens and the resulting compound (glycoyamine) gives the Sakaguchi reaction.



These findings are of considerable interest in view of the recent work that has been done by Goldblatt, *et al.* (1) who first produced chronic arterial hypertension without loss of urea clearance in animals by application of adjustable clamps to the main renal arteries. The later work of Page, *et al.* (1) shows the following reaction occurs in the production of experimental hypertension:

Renin + renin activator \rightarrow Angiotonin + angiotonin activator \rightarrow inactive substance. Angiotonin causes marked elevation of blood pressure both in dogs and in men. Page, *et al.* (1) have isolated this substance from kidney tissue and have shown it to be a compound which gives the Sakaguchi reaction for arginine. This reaction is also given by glycoyamine, methyl guanidine, agmatine or arcaine. Since angiotonin gives picrates and oxalates it must be a nitrogenous base. Its solubility in alcohol, however, rules out arginine and glycoyamine as being the active factors in raising the blood pressure. Page, *et al.* (1) also stated that Weber's reaction for guanidine like substances was negative when tested with

angiotonin. It is possible that this active factor is a new guanidine base which also gives the Sakaguchi reaction.

Arkin, *et al.* (1) have used the determination of plasma creatinine level as a test of low grade kidney damage. In kidney damage the glomerular function is usually the first to

TABLE 4a

PLASMA-CREATININE VALUES IN PATHOLOGICAL CASES
(After Arkin, *et al.* (1))

Diagnosis	Num- ber of Ex- amina- tions	Maxi- mum Creati- nine, mg. %	Mini- mum Creati- nine, mg. %	Aver- age Creati- nine, mg. %	PERCENTAGE OF CASES WITH BLOOD CREATININE		
					Below 1 mg. %	1 to 1.4 mg. %	Above 1.4 mg. %
Acute nephritis	26	56	1.44				
Extraglomerular acute nephritis	1	2	37.10	37.10	37.10	0	0
Transitional stage between acute and chronic nephritis	3	3	2.60	1.33	2.16	0	33.3
Acute exacerbation of chronic nephritis	2	5	24.80	1.14	9.36	0	0
Chronic nephritis	33	67	26.08	1.01	6.06	0	18.2
Nephrosis	12	27	2.60	.62	1.32	16.7	66.7
Amyloidosis	1	1	1.76	1.76	1.76	0	0
Benign nephrosclerosis	73	115	4.20	.49	1.24	34.2	34.2
Malignant nephro- sclerosis	24	43	17.10	1.15	4.18	0	8.3
Polycystic kidneys	1	3	1.99	1.89	1.94	0	0
Obstruction of urinary passages	19	40	15.85	.80	4.95	15.8	15.8
Pyelitis	9	12	1.07	.76	.89	77.8	33.2
Extra-renal azotemia heart failure	26	35	1.91	.70	1.05	38.2	46.3
Pneumonia	5	5	1.22	.45	.98	60.0	40.0
Liver disease	27	34	16.52	.56	2.15	33.3	29.6
Hypochloremia	9	15	7.60	.76	1.95	11.1	33.4
Diabetic acidosis	5	5	4.28	1.04	2.58	0	20.0
Miscellaneous	2	6	3.36	.93	1.50	0	50.0
Total	278	474					

be impaired as indicated by reduction of glomerular filtration. The original method of Rehburg (1) who used the exogenous creatinine clearance as a measure of glomerular function was discarded for reasons similar to those discussed in Chapter XI on the Creatinine Clearance.

According to Arkin, *et al.* (1) *endogenous creatinine* fol-

lows a different metabolic path from *exogenous creatinine* (Cf. Chapters VI and IX). The former type of creatinine is filtered through the glomerular loops and is probably, under normal conditions, neither secreted nor absorbed in the tubular part. Hence any disturbances of glomerular filtration

TABLE 4b

CORRELATION BETWEEN PLASMA CREATININE AND BLOOD PRESSURE OR ALBUMINURIA IN PATHOLOGICAL CASES. THE HYPERTENSIVE KIDNEY DISEASES ARE SEPARATED FROM THE OTHER CASES

(After Arkin, *et al.* (1))

	Number of Cases	Maximum Creatinine	Minimum Creatinine	Average Creatinine
<i>Hypertensive</i>				
B.P. below 150	1	1.02	1.02	1.02
B.P. between 150-180	7	2.32	1.12	1.40
B.P. between 180-220	28	17.1	.59	3.27
B.P. above 220	11	15.0	1.08	4.00
Urine—0	9	2.50	.59	1.82
Urine—One Plus	15	8.35	.60	2.34
Urine—Two Plus	9	11.30	1.15	2.56
Urine—Three Plus	14	17.10	1.02	4.66
<i>Non-Hypertensive</i>				
B.P. below 150	40	16.52	.45	2.93
B.P. between 150-180	12	10.85	.69	2.52
B.P. between 180-220	14	24.80	.78	4.76
B.P. above 220	4	26.08	2.03	10.07
Urine—0	14	4.22	1.01	1.47
Urine—One Plus	18	16.52	.45	3.70
Urine—Two Plus	15	8.45	.62	2.43
Urine—Three Plus	24	26.08	.86	5.48

should cause a retention of creatinine in the blood. The glomerular filtration undergoes physiological variations while the blood creatinine remains fairly constant. For clinical purposes, therefore, the blood creatinine determination gives as much, if not more, information than the determination of glomerular filtration. A mild reduction of the urea filtration can be compensated by reduction of the physiological urea re-

absorption in the tubules. A reduction in the creatinine filtration cannot be compensated (*Cf.* Chapter XI).

Arkin and coworkers made 474 plasma creatinine determinations on 278 patients. Their results are listed in Table 4a.

The plasma creatinine usually runs parallel with the blood pressure and degree of albuminuria (Table 4b).

According to Arkin the clinical value of the plasma creatinine determination lies in the low ranges; the increase probably indicates a slightly disturbed glomerular filtration. The test in these ranges is superior to the determination of non-protein nitrogen and urea not only for theoretical reasons (lack of reabsorption in the tubules), but also because of the sharper limits between normal and pathological ranges, and because of the smaller influence of extrarenal factors such as alimentary food intake or protein breakdown. The demonstration of these disturbances is of diagnostic and prognostic importance in acute and chronic nephritis, nephrosis, nephrosclerosis, urologic conditions and heart failure.

CHAPTER V

FORMATION AND EXCRETION OF GLYCOCYAMINE

IN SPITE of the fact that glycoyamine, or guanidine acetic acid, has long been known to be methylated to creatine in the animal body, its rôle as a creatine precursor was not generally accepted until recently. Only a small amount of it was supposed to be methylated per day and it had never been isolated from animal tissues or fluids. Weber (1, 2, 3) was the first to isolate it from human and dog urine. Weber (4, 5) introduced his method for the determination of glycoyamine in 1930. It was modified by Bodansky (10) and by Davenport and Fisher (5). Dubnoff and Borsook (7) stated that the method of Davenport and Fisher was laborious and time consuming, the adsorption of glycoyamine on Lloyd's reagent was incomplete, further losses of glycoyamine occurred in the repeated treatment with permutit (these losses were 10 per cent by the English workers and 80 per cent by Dubnoff and Borsook) and the color developed was unstable. As the amount of arginine decreased the loss of glycoyamine increased. These disadvantages were removed in the method for the determination published by Dubnoff and Borsook (7). Added glycoyamine was recovered quantitatively and their method is highly accurate.

Borsook and Dubnoff (4), using their kidney slice tech-

nique and their method for determination of glyco-*cy*amine, (Dubnoff and Borsook (7)) showed that arginine and glycine formed glyco-*cy*amine in the presence of kidney slices and the enzyme bringing about this reaction was named "glycine-transamidinase." Its optimum pH was 7. This transamidination did not occur in the presence of slices of liver, heart, or skeletal muscle, blood, brain or spleen. Glyco-*cy*amine was also formed in the kidney slices from citrullin and glycine and from arginine and sarcosine.

TABLE 5
EFFECT OF INGESTION OF 65 GM. OF GELATIN ON
URINARY GLYCO-*cy*AMINE
(After Borsook, *et al.* (4, 5))

Subject	Non-protein Day Glyco- <i>cy</i> amine mg.	Gelatin Day Glyco- <i>cy</i> amine mg.	Increase per cent
1	43.7	85.7	
2	34.3	114.0	
3	34.0	60.0	
4	32.9	88.2	
5	24.4	81.6	
Average	33.5	86.3	157.0

Using their sensitive method, glyco-*cy*amine was found to be widely distributed in the different tissues of the rat. The concentrations in mg./100 gm. were as follows: brain 0.5 to 1; heart, liver, skeletal muscle and spleen 3 to 6; small intestine 10; kidney cortex 15 to 30. It was stated that the rates of glyco-*cy*amine production observed in their studies were sufficient to make good the total loss of creatine and creatinine from the tissues. This statement was based upon the not unreasonable assumption that the rate of glyco-*cy*amine formation in the human kidney *in vivo* is of the same order of magnitude as in rat kidney slices *in vitro*. The above reaction was catalyzed by cell free extracts of kidney as well as in kidney slices.

It was found by Borsook, *et al.* (4, 5) that the formation of glycocyamine from the amino acids together (arginine

TABLE 6

EFFECT OF FEEDING PROTEINS AND AMINO ACIDS UPON CREATININE ELIMINATION IN MAN
(After Beard and Barnes (3))

Subject	Average Control Creatinine Excretion	Experimental Creatinine Excretion	Increase per cent	Meat-free Diet Plus
	gm.	gm.		
H.H.B.	1.63	2.00	22.0	100 gm. casein
	1.63	2.60	58.5	100 gm. casein
	1.63	2.00	22.0	100 gm. edestin
	1.63	2.00	22.0	100 gm. fruit jello
	1.63	1.94	12.2	(2nd day)
	1.63	2.07	26.2	8 gm. glycine
	1.63	2.40	46.3	(2nd day)
	1.63	1.98	44.5	(3rd day)
	1.63	2.07	26.8	(4th day)
	1.63	2.56	56.1	8 gm. glycine
	1.63	1.44	0.0	8 gm. glycine
	1.63	2.24	37.2	10 gm. alanine
	1.63	1.98	20.7	(2nd day)
	1.63	2.25	37.3	(3rd day)
	1.63	1.95	12.8	(4th day)
	1.63	2.01	22.5	5 gm. arginine HCl
	1.63	2.33	42.1	(2nd day)
	1.63	2.21	34.7	5 gm. arginine HCl
	1.63	1.91	16.4	(2nd day)
G.D.M.	1.17	1.90	62.4	100 gm. casein
	1.17	1.31	11.9	100 gm. edestin
	1.17	1.39	18.7	100 gm. fruit jello
G.G.	1.33	1.95	47.3	8 gm. glycine
	1.33	1.80	35.3	8 gm. glycine
R.F.H.	1.77	1.96	10.7	10 gm. glycine
	1.77	2.05	15.8	10 gm. glycine

and glycine) was greater than could be observed from each alone. This was also true when gelatin was fed to man. The California workers also admit the possibility of creatine

formation from amino acids other than glycine and arginine. This is further proof against the theory of Schoenheimer, *et al.* discussed in Chapter VI, that glycine and arginine are the only amino acids that take part in creatine synthesis in the body.

Borsook's findings that within 2 hours after feeding gelatin or ingesting arginine and glycine by 7 normal men, there is an average increase of 157 per cent (in the gelatin experiment) in glycoxyamine excretion in the urine, offers much additional proof of creatine formation from these amino acids or gelatin. Since there is also a large creatine and creatinine formation from these amino acids under their experimental conditions, it is certain that much more glycoxyamine is formed in Borsook's experiments in man than is accounted for by his findings, since it would be only the retained glycoxyamine that would be transformed into creatine and creatinine under these conditions. Compare Table 5 from Borsook (5) and Table 6 from Beard and Barnes (3).

At the present time there are no available data on the formation of glycoxyamidine in metabolism. Nevertheless, the possibility remains that this anhydride of glycoxyamine may be formed from the amino acids in an analogous way to the formation of glycoxyamine. The writer is of the opinion that glycoxyamidine is methylated to creatinine in the same way as glycoxyamine is methylated to creatine in the body (11, 14).

CHAPTER VI

ORIGIN OF CREATINE

SINCE CREATINE is the chief nitrogenous constituent of muscle tissue, its origin and function have interested investigators for many years. In spite of the fact that the nitrogen of creatine must originate from the nitrogen of the tissues, and that it was always generally believed that creatine originated from the protein of the diet, the conclusions drawn by Folin, Rose, Thomas, Hunter and many others from investigative work in this field, served from 1912 until 1925 to cast doubt on the exogenous origin of creatine and creatinine. To quote:

"Believing, as they (Folin and Denis (6)) do that creatine exists only as an integral part of living protoplasm, they point out that it is not possible to increase the mass of tissues by feeding and that it would therefore be almost hopeless to attempt to trace the formation of creatine to any constituent of the food."—Hunter (1). Creatine is "an *anabolic* product formed for a specific purpose or function and its formation proceeds only so far as it is needed by the cells"—(Rose, Dimmitt and Bartlett (1)). "Its (creatine) rate of production is therefore in all likelihood regulated by an internal demand, and it is not to be expected that it should be accelerated by an exogenous supply of precursors, any more than the production of adrenaline or thyroxine would be increased by the administration of a dose of tyrosine."—Hunter (1).

These views led to the acceptance of the concept that creatine and creatinine were substances *entirely of endogenous origin* and the many successful attempts that were made to show that this view was untenable were not generally accepted even up to the present time. Let us discuss briefly some of the evidence for these theories.

Folin (1, 2, 3) published his classical theory of protein metabolism in 1905. The data which were used as the basis of this theory were obtained by feeding diets rich and low in protein to normal individuals. The exogenous metabolism yielded chiefly urea and inorganic sulfates, no creatinine and probably no neutral sulfur, and was very variable, while the endogenous, or constant metabolism, was independent of the exogenous metabolism and was represented largely by the *excretion of creatinine* and neutral sulfur. Thus the idea became general that creatinine excretion was uninfluenced by the diet, and, since creatine was supposed to be the mother substance of creatinine, both of these substances were considered to be of endogenous origin in the body.

Mitchell and Hamilton (1) gave a good discussion of these theories of Folin. Further work by Borsook and Keighley (7), Bloch and Schoenheimer (1) and Schoenheimer, *et al.* (10) casts considerable doubt on Folin's conception of a distinction between the exogenous and endogenous metabolism. His conception of the endogenous metabolism rested largely on the constancy of the creatinine excretion and on the fact that its excretion was not influenced by diet. In recent years it has been shown by several workers that, while the creatinine excretion does not seem to be influenced by the ingestion of a normal protein diet, nevertheless, the feeding of large quantities of proteins, such as casein or gelatin, or the amino acids such as glycine or arginine, may in many cases increase the creatinine and creatine excretion (*Cf.* Chapter VIII and glycocyamine excretion, Chapter V).

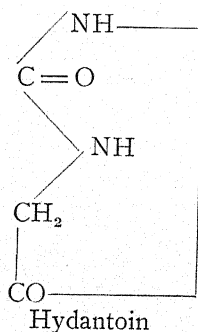
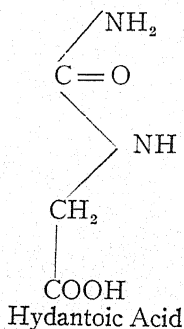
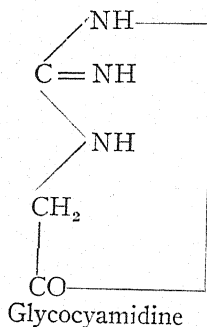
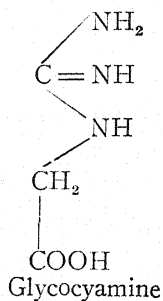
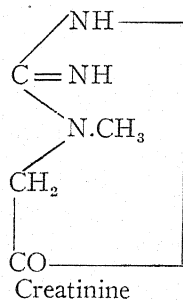
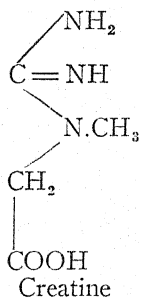
It was also supposed that (since the creatinine excretion was constant and since it arises from the creatine of the muscle) the muscle creatine would also be constant under usual dietary conditions. Reference to the data published in Hunter's monograph will show, however, variations as much as 50 to 100 per cent in the concentration of the muscle creatine in various muscles of different species of animals. These variations can only be due to the influence of different types of diet ingested.

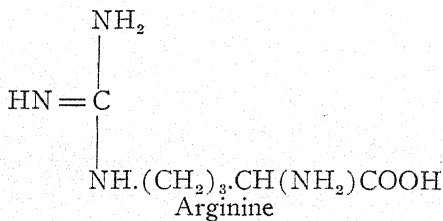
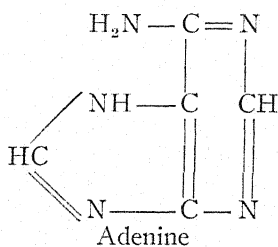
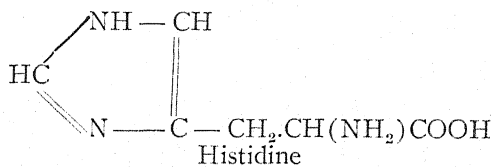
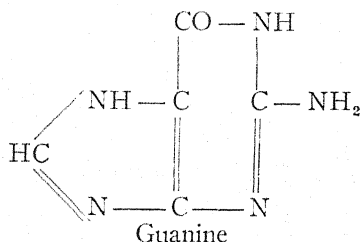
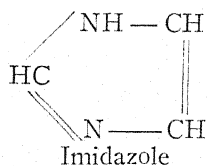
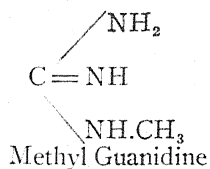
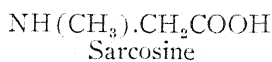
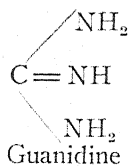
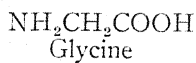
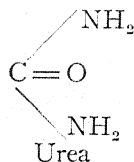
There was also a good deal of negative evidence published in the past, showing that ingestion of the amino acids would not increase the concentration of muscle creatine in animals. Most of these creatine determinations were made before the introduction of the method of Rose, Helmer and Chanutin (2). Not enough of the amino acids was given and sufficient time was not usually allowed to obtain increased creatine formation. *A normal protein diet should be fed* to the experimental animals or man if increases in muscle creatine are to be obtained after ingestion of the amino acids. For instance, these criticisms apply to the studies reported by Bodansky (1, 2), Hyde (1) and others. The amino acids undergo several different types of biochemical reactions in the body and only two of these are employed in the formation of creatine and creatinine.

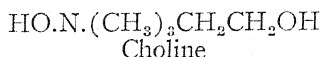
The author made an extensive study of the literature of creatine and creatinine metabolism from 1919 to 1922, and came to the conclusion that several of the concepts of this period had not met with universal acceptance. The studies of Thompson (1, 2) showing that arginine increased muscle creatine and creatine excretion in several species of animals were very impressive; and Gross and Steenbock (1) and Crowdle and Sherwin (1) showed the same effects when arginine was fed to pigs and chickens. Hunter (1) attributed Thompson's results to a general amino acid stimulation

which, however, is not the correct interpretation of his findings at the present time.

Before discussing the results of our studies on the origin of creatine and creatinine let us compare the structural relationships of these with several closely related compounds, as follows:







Creatine is methyl guanidine acetic acid and creatinine is its anhydride, or methyl glycoyamidine. Thus creatine may appear as a derivative of glycine (amino acetic acid), sarcosine (methyl amino acetic acid), guanidine, methyl guanidine, glycoyamine (guanidine acetic acid), and hydantoin (uramino acetic acid). If creatine is regarded as a derivative of glycine, it will be closely connected with the whole group of natural amino acids which occur in the protein molecule from which these amino acids take origin. Its guanidine group, however, links it with the amino acid arginine, which is also a guanidine derivative. Its N-methyl group is distinctive and brings it into relation with the betaines and choline. The ring closure, which converts creatine to creatinine (or the opening of the ring which occurs when creatinine is transformed into creatine), makes creatinine a homologue of glycoyamidine and this brings it in relation to hydantoin. These substances may be derived from a hydrogenated imidazole, and this connects creatinine to the amino acid histidine, the purines, methyl purines and allantoin. Guanine, among the purines, has two relations with creatinine, through the imidazole nucleus which is common to all purines, and as part of its pyrimidine ring, a guanidine residue.

This is also true of the purine adenine, which is a component of the nucleotide, adenylic acid (adenine, ribose and phosphoric acid). On hydrolysis of the nucleotide, the phosphoric acid is split off leaving the nucleoside, adenosine, which, as adenosine di- and triphosphates, plays an important rôle with creatine phosphate in the chemistry of muscular contraction (*Cf.* Chapter XIV).

The close chemical relations of creatine and creatinine with these substances will be shown, in the following discussion,

to hold also from the physiological point of view, since it will be shown that practically all of them (all of those tested) will serve to increase either creatine formation in the muscles or creatine-creatinine excretion in the urine.

The writer decided to make a systematic study of various aspects of this type of metabolism beginning in 1925 in

TABLE 7

INFLUENCE OF FEEDING PROTEINS, AMINO ACIDS AND RELATED SUBSTANCES UPON CREATINE FORMATION IN RAT MUSCLE*
(After Beard (2))

<i>Substance Fed</i>	<i>No. of Rats</i>	<i>Creatine, per cent</i>	<i>Increase Over Controls, per cent</i>
Glycine	9	0.46	15.0
<i>dl</i> -Alanine	17	0.45	12.5
<i>dl</i> -Valine	6	0.54	35.0
<i>d</i> -Glutamic Acid	8	0.48	20.0
<i>l</i> -Aspartic Acid	10	0.47	17.5
<i>l</i> -Cystine	10	0.55	37.5
Histidine	4	0.49	22.5
<i>dl</i> -Phenylalanine	5	0.49	22.5
<i>l</i> -Tyrosine	10	0.49	22.5
<i>d</i> -Arginine-HCl	11	0.51	27.5
<i>l</i> -Leucine	4	0.49	22.5
Choline-HCl	6	0.49	22.5
Glycocyamine	3	0.59	47.5
Casein	6	0.52	30.0
Edestin	6	0.52	30.0
Creatine	8	0.49	22.5

* The average muscle creatine of 118 comparable control animals was 0.40%.

Cleveland at Western Reserve University School of Medicine. Our first study showed, contrary to the teachings of Folin and others, that creatinine output was not influenced by the body weight of the individual (Beard (1)). A short time later definite evidence was obtained in preliminary studies showing that both creatine and creatinine were formed by feeding amino acids in the diet (Beard (2)). These results are listed in Tables 7 and 8.

TABLE 8

INFLUENCE OF FEEDING AMINO ACIDS UPON
CREATININE ELIMINATION IN THE RAT
(After Beard (2))

<i>Amino Acid Fed</i>	<i>No. of Exps.</i>	<i>Average Increase in Excretion of Creatinine, per cent</i>
Glycine	21	35.9
<i>l</i> -Aspartic Acid	11	14.2
<i>d</i> -Arginine-HCl	7	26.8
<i>dl</i> -Alanine	8	28.0
Histidine	6	19.5
<i>l</i> -Cystine	8	28.1
<i>dl</i> -Valine	5	14.8
<i>l</i> -Tyrosine	8	30.5
<i>d</i> -Glutamic Acid	9	25.1

So unusual and unexpected were these results that their final publication was held up for over five years until more data could be obtained upon the subject. These results show, however, that there is practically a *uniform increased formation of creatine and excretion of creatinine when the amino acids and related substances are fed to young rats.*

Rose (3) in his review of the subject in Volume 2 of the Annual Review of Biochemistry referred to the above results as follows:

"It is evident from what has gone before that our knowledge of the origin of creatine (or creatinine) is in a very unsatisfactory state. But if one is to accept seriously the paper of Beard and Barnes (3), the situation becomes chaotic. These investigators report that the creatine content of the muscles of young rats may be increased by the feeding of arginine-HCl, etc. . . . The amino acids listed are said to augment greatly the output of creatinine in the urine of adult rats; and, in so far as they were tested, in human subjects as well. Without questioning the accuracy of the author's observations, one is justified in assuming that the results are not to be attributed to a direct transformation of the compounds into creatine and creatinine. To make any

other assumption would be quite unreasonable, it seems to the writer, in view of the wide dissimilarity of the compounds fed. The doses were extraordinarily large amounting in most cases to 1 gm. for a 40 to 55 gm. rat. In such proportions the acids may have exerted toxic effects. Furthermore, in order to induce the animals to consume the amino acids, the food cups were removed from the cages for several hours. Young rats are very sensitive to starvation, and the creatine-creatinine changes may have been due to this factor. But whether the foregoing suggestions suffice to account for the findings of Beard and Barnes or not, the apparent stimulation in creatine formation and creatinine elimination certainly must have been of a non-specific character."

Let us examine some of these criticisms. An adult rat will eat about 10 gm. daily of a diet containing 20 per cent of protein. (Even the consumption of diets high in protein for some time does not necessarily damage the kidneys.) They would, therefore, consume from 2 to 3 gm. of protein per day, containing at least 2 gm. of mixed amino acids. The feeding of 1 gm. or injection of 100 mg. of a single amino acid could not, therefore, be considered "extraordinarily large" as stated by Rose. Furthermore, an examination of the kidneys of our rats (Beard and Barnes (3)) by Doctor Moore of the Institute of Pathology of Western Reserve University did not show any pathology resulting from the ingestion of the 1 gm. of the amino acids. The few hours in which the diet was removed from the cage could not have been the cause of the increases in creatine and creatinine observed.

The discovery of phosphocreatine in 1927 by Fiske and Subbarow (1) and the Eggletons (1) and of the rôle it plays in muscle contraction (Lundsgaard (1)), followed by the publication of "The Revolution in Muscle Physiology", by A. V. Hill (1) served to again emphasize the importance of creatine in the body, and soon thereafter many efforts were made to discover the origin of this interesting substance.

Brand, *et al.* (1) at the meeting of the International Physiological Congress in Boston in 1929 delivered a paper on the origin of creatine, which served to create further interest in the subject. The New York investigators showed that the feeding of glycine to patients suffering from progressive pseudohypertrophic muscular dystrophy caused an increase up to 40 per cent in creatine excretion. From this observation they drew the conclusion that glycine was a precursor of creatine. While it is definitely known today that this statement is correct, nevertheless these data of Brand could not be considered in 1929 to show the *origin* of creatine from glycine. It is well known that there are large *variations* in creatine excretion in the myopathy patient without any therapy and creatinuria may be produced by a number of factors not related to the muscular system at all. Their data only showed that glycine did increase the creatinuria slightly, but this observation was not accepted at the time as proving the origin of creatine from glycine.

Numerous studies have been conducted in the author's laboratory during the last 18 years on the origin of creatine and creatinine. Beard (2); Beard and Barnes (3); Beard and Boggess (4, 28, 33); Tripoli and Beard (5, 8); Tripoli, McCord and Beard (6); Beard, Tripoli and Andes (7); Beard (9); Boggess and Beard (10); Beard and Pizzolato (11); Beard, Espenan and Pizzolato (12); Beard (13); Kelly and Beard (14); Beard and Jacob (17); Beard, *et al.* (22). The diet fed to the experimental animals throughout was composed of 2/3 whole wheat flour, with 1/3 whole milk powder, together with 1 per cent of the weight of the wheat, each, as NaCl and CaCO₃ (*i.e.*, 100 gm. of wheat flour with 1 gm. of NaCl and 1 gm. of CaCO₃). Animals from more than 60 generations have been used in these studies, ranging in weight from 100 to 150 gm. The injection of a supposed precursor of creatine or creatinine is usu-

ally followed by a study of the excretion of these substances in the urine. When this study is completed the same animals are reinjected with identical amounts of the same substance and the muscle creatine determined. The study is usually repeated three times, using a sufficient number of animals to obtain reproducible results. The modifications of the Jaffé reaction discussed in Chapter II were used in all of our studies after 1936. The Miller and Dubos specific creatinine enzyme was used when deemed necessary. The total number of animals used to date in these studies is well over 3,000, and the average muscle creatine of over 500 control animals was 0.42 ± 0.03 per cent.

The author has submitted the following reactions to illustrate the synthesis of creatine and creatinine *in vivo* and *in vitro*.

- I. *Glycine + Urea* \rightarrow (*Hydantoic Acid*) \rightarrow *Glycocyamine* \rightarrow *Creatine*.*
- II. *Sarcosine + Urea* \rightarrow *Creatine and Creatinine*.
- III. *Glycine + (Cyanic Acid)* \rightarrow (*Hydantoic Acid*) \rightarrow *Glycocyamine* \rightarrow *Creatine*.
- IV. *Sarcosine + (Cyanic Acid)* \rightarrow (*Methyl Hydantoic Acid*) \rightarrow *Creatine*.
- V. *Arginine* \rightarrow (*Guanidine Butyric Acid*) \rightarrow *Glycocyamine* \rightarrow *Creatine*.
- VI. *Urea + Methyl Amine* \rightarrow *Methyl Guanidine* \rightarrow *Creatine*.
- VII. *Creatinine + Water* \rightarrow *Creatine*.

Reactions I, II, V, and VII occurred under our experimental conditions, while the rest are of much theoretical interest. Let us now list those cases from the literature which show the validity of these reactions.

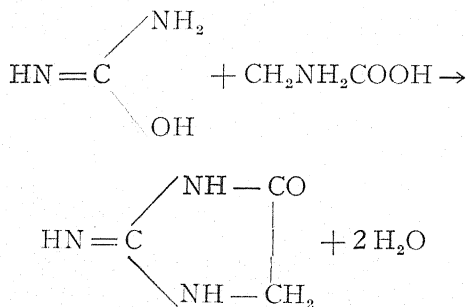
* Methyl urea can also be used in this reaction instead of urea.

Reaction I.

a. Werner (1) has produced much evidence for his "cyclic" formula for urea as compared to the older "carbamide" formula.

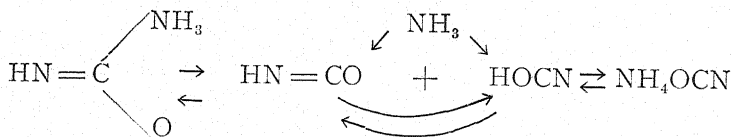
b. Urea + Glycine (heated in a sealed tube with glycerine) \rightarrow Glycoeyamidine (Sasaki (1)).

Two moles of water are displaced by melting urea and glycine in a sealed tube with glycerine at a high temperature. Sasaki gives the following equations to illustrate how this is possible.

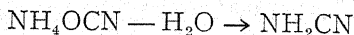


The mechanism of the reaction can be explained as follows:

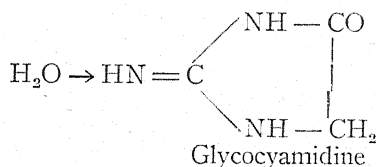
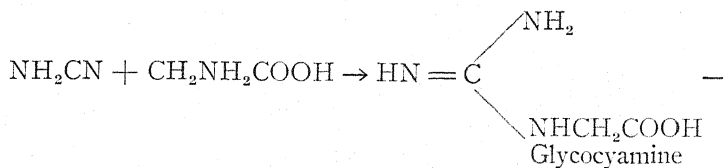
First—On application of heat, the equilibrium between urea and ammonium cyanate arises in glycerine,



Ammonium cyanate changes to cyanamide after dehydration at the high temperature,



Second—The cyanamide thus formed combines with glycoll and then produces glycoyamine which is further transformed into glycoyamidine at the high temperature,



Since creatine or creatinine is the methylated product of glycoyamine or glycoyamidine, Sasaki suggests that the formation of creatine or creatinine in the human body may also occur from the synthesis of glycoyamine or glycoyamidine from urea and glycoll as in the above reaction. It would seem, however, that this reaction is much too drastic to occur in the human body. Nevertheless the author has shown in several studies that it does take place *in vivo* and *in vitro* (see below).

c. Urea + Glycine \rightarrow Glycoyamine (Beard and Espenan (21)). (Methyl Urea + Glycine \rightarrow Creatine (Beard, unpublished observations)).

d. Glycoyamine \rightarrow Glycine + Urea, and the enzyme glycoyaminate in the liver produces the same change (Mathews (1)).

e. The body can conjugate carbamic acid and some amino acids to form uramino acids (Mathews (1)).

f. Guanidine + Glycine \rightarrow Glycoyamidine (Wheeler and Merriman (1)).

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g. Urea and glycine are products of protein metabolism and the amino group of the amino acid is necessary for creatine synthesis (Beard and Pizzolato (11)).

h. Guanidine + Glycine Ester \rightarrow Glycocyamidine (Traube and Ascher (1)).

i. Potassium Cyanate or Urea + Glycine \rightarrow Carbamido Acids + HCl \rightarrow Hydantoins (Boyd (1)).

Reaction II.

a. Sarcosine + Guanidine Carbonate \rightarrow Creatine + Creatinine (Paulman (1)).

b. Sarcosine + Thiourea \rightarrow Creatine (King (1)).

c. Sarcosine Ethyl Ester + Guanidine \rightarrow Creatine (Abderhalden and Sickel (1)).

d. Sarcosine Ethyl Ester + Triacetyl-anhydroarginine \rightarrow Creatine (Bergmann and Zervas (1) suggested many years ago the transfer of the amidine group (transamidination) from arginine to guanidine acetic acid. Triacetyl-anhydroarginine easily yields this group to glycine with subsequent formation of glycocyamine and creatine).

(BaOH₂)

e. Creatine $\xrightarrow{\quad\quad\quad}$ Sarcosine + Urea (Liebig (1)). This reaction shows, according to Schmidt (2) that creatine may be considered as the ureide of sarcosine.

f. Guanidine Carbonate + Sarcosine \rightarrow Creatinine (Horbacewski (1)).

g. Sarcosine + Urea \rightarrow Creatine (Beard and Espenan (21)).

Reaction III.

a. This is a well known method of preparation of hydantoic acid from cyanic acid.

b. Werner (1) suggested that urea may be produced from cyanic acid in the body.

c. Cyanate is present in the blood of the cat and rabbit (Montgomery (1)).

d. Cyanate occurs in the blood of the dog (Gottlieb (1)).

e. Urea + Glycine (120° C.) \rightarrow Hydantoic Acid (Lipich (1)).

f. Potassium cyanate + Glycine Ethyl Ester HCl \rightarrow Hydantoic Acid (Harries and Weiss (1)).

g. Amino acids contain the following groups ($-\text{H}_2\text{C}-\text{NH}_2$; $=\text{C}-\text{NH}_2$; $=\text{HC}-\text{NH}-$) which could readily yield $\text{O}=\text{C}=\text{NH}$ as the final stage in their oxidation (Werner (1)).

h. Fifty mg. of KOCN increased creatine formation in the muscles of the rat (Beard and Pizzolato (11)).

i. Cyanamide + Glycine \rightarrow Glycocyamine (Strecker-Volhard synthesis, Hunter (1)).

j. Cyanamide + Glycine \rightarrow Glycocyamine (Beard and Espenan (21)).

k. Cyanamide is formed *in vitro* by the oxidation of sugars in the presence of ammonia, and formaldehyde is an intermediate product of the reaction (Fosse and de Larambergue (1, 2)).

Reaction IV.

a. Cyanamide + Sarcosine \rightarrow Creatine (Volhard (2)).

b. Cyanamide + Sarcosine \rightarrow Creatine (Beard and Espenan (21)). The addition of a few drops of strong ammonia greatly speeds up the rate of this reaction.

c. Creatine + $\text{Ba}(\text{OH})_2 \rightarrow$ Sarcosine + Urea (Liebig (1)) \rightarrow Methyl Hydantoic Acid (Gaebler (2)).

d. Urea + Sarcosine \rightarrow Methyl Hydantoic Acid (Gaebler (2)).

e. Sarcosine \rightarrow Methyl Hydantoic Acid + Oxalic Acid (Schultzen, Cf. Mathews (1)).

f. "The cyanogen of the Strecker-Volhard synthesis attaches itself to glycoll or sarcosine, not through the hydroxyl but through the amino or methylamino group, and

creatine and glycoyamine are not substituted ureas but substituted guanidines. The possibility of such a mode of combination was proved by Erlenmeyer through his synthesis of guanidine-HCl by the action of cyanamide upon ammonium chloride in alcoholic solution at 100° C. The formation of creatine in Volhard's synthesis is exactly analogous." —(Hunter (1)).

Reaction VI.

a. Urea + Methyl Amine \rightarrow Methyl Guanidine + H₂O
(Dessaigues (1)).

b. Creatine, Sarcosine, or Methyl Guanidine $\xrightarrow{\text{NaOH}}$
Methyl Amine (Dessaigues (1)).

c. Sarcosine + Lead Peroxide \rightarrow Methyl Amine (Dessaigues (1)).

d. Creatine + HNO₃ \rightarrow Methyl Amine (Dessaigues (1)).

e. Monochloroacetic acid + Methyl Amine \rightarrow Sarcosine
(Volhard (1)).

f. Sarcosine Ethyl Ester + Guanidine \rightarrow Creatine (Abderhalden and Sickel (1)).

g. Glycine \rightarrow Methyl Amine (Kohn (1)).

h. Creatine \rightarrow Methyl Amine (Kapellar-Adler and Toda (1)).

i. Creatine + HgO \rightarrow Methyl Guanidine + Oxalic Acid
(Mathews (1)).

The results from the author's laboratory in regard to the origin of both creatine and creatinine may be summarized as follows:

1. The feeding of proteins or amino acids, except proline and hydroxyproline, or the injection of amino acids and related substances, increases creatine formation in rat muscle (2, 3, 4, 10, 12, 13, 23, 28, 33), dog muscle (9), and crea-

tine-creatinine excretion in the rat (3, 10, 12, 13) and man (5, 6, 7, 8, 12, 13).

2. Creatine formation under these conditions is not due to the specific dynamic action of the amino acids (3).

3. The amino acids may be metabolized through urea and glycine in creatine synthesis (11). (Ratner, *et al.* (3) showed that glycine, as well as other amino acids, could both donate and accept nitrogen (transamination) from each other in protein metabolism.)

4. The presence of the amino group of the amino acid is necessary for creatine synthesis (3, 11) and the presence of a few drops of strong ammonia greatly increases the speed of creatine formation from methyl urea and glycine and from cyanamide and sarcosine (21).

5. The guanidine group of creatine is synthetic in origin and can be formed from arginine, urea, creatinine, glyco-cyamidine or glyco-cyamidine by the process of the "guanidine shift" (3, 5, 10, 11, 12, 13).

6. Increasing the methylation process in the body results in increased creatine formation and excretion (14, 15, 16).

7. Creatine is usually formed from the proteins and amino acids and its synthesis is not limited to the physiological requirements for it but depends upon the amount of its precursors in the diet (11).

8. Creatine formed from the amino acids is stored in the rat for 2 to 4 days only (3, 4, 11). This is also probably true for man (31).

9. Injection of urea with glycine, sarcosine, betaine, or choline, gives over twice as much extra creatine formation in young rats as the injection of the same amounts of these substances alone (11).

10. Urea and glycine can each be synthesized in creatine formation in the rat (12).

11. Amino acids may also serve as sources of urea in this connection (11, 12).

12. Glycine peptids, sarcosine and glutathione may act as sources of glycine and hydantoin may supply both urea and glycine (11).

13. The extent of creatine formation and excretion probably depends upon the amount of available phosphate present in the tissues (22, 31).

14. Injection of small doses of the purines and their methylated derivatives (15, 16) or of 1 gm. of creatinine (17) *greatly stimulates* the formation and excretion of both creatine and creatinine in the urine.

15. The injection of creatinine and water (or physiological saline) together gives twice as much extra creatine excretion as compared to the injection of either alone. The same was true in the case of water as compared to saline (22).

16. The body metabolizes administered creatine and creatinine in a different manner from creatine and creatinine formed in normal amino acid metabolism. This is shown as follows: Administered creatine is of little value to the myopathic individual and is largely excreted as such (5, 6, 7, 8), the remainder probably being oxidized in the body; it does not increase the energy output in students when it is ingested (31) while creatine formed from the amino acids or from creatinine is changed into creatine phosphate which plays an important rôle in muscular contraction (27, 31), (Cf. E. Wang (1)). Pitressin or pitocin causes a retention of *body* creatinine which is followed by its quantitative transformation into creatine. On the other hand these hormones have no effect upon *administered* creatinine (26). (Our work on the relation of the hormones to creatine-creatinine metabolism is summarized in Chapter XIII.)

Schoenheimer, *et al.* (Bloch and Schoenheimer (1, 2, 4, 5,

6, 7)), Ratner, Schoenheimer and Rittenberg (3), Foster, Schoenheimer and Rittenberg (8), Rittenberg, Schoenheimer and Keston (9), Schoenheimer, Ratner and Rittenberg (10), and Schoenheimer and Rittenberg (11) have also made very important contributions to the question of the origin of creatine and to the metabolism of the amino acids in the body using their tracer technique. Ammonia containing N^{15} can be utilized by the rat and can, in part, be transformed into creatine containing N^{15} (7, 8, 9, 10, 11). Glycine, as well as other amino acids, can donate and accept nitrogen (transamination) from each other in protein metabolism (3). Several amino acids and related substances, *dl*-tyrosine, *l*-leucine, sarcosine, glycine, arginine, and glyco-cyamine, each containing N^{15} can be transformed into creatine containing N^{15} (7, 8, 9, 10, 11). After feeding arginine containing N^{15} , a large amount of creatine containing N^{15} was isolated, showing that the amidine group of creatine must have arisen from the amidine group of arginine ("amidine shift"), which has also been shown by the New York investigators to be continuously formed in normal animals from the α amino nitrogen of various amino acids. The nitrogen of the amidine group of creatine can also be obtained from other amino acids, while glycine and sarcosine serve to increase the glycine moiety (portion) of creatine (1, 7). The ingestion of sarcosine containing N^{15} leads to the deposition in proteins of glycine containing N^{15} to the same extent as when glycine is fed. It was stated, therefore, that sarcosine does not appear to be an intermediate in creatine formation, but leads to its production through demethylation to glycine (1, 4, 7), a reaction also observed by Abbott and Lewis (1), Handler, *et al.* (2) and Borsook and Dubnoff (4).

Schoenheimer is of the opinion that the amino acids besides glycine and arginine form creatine through their ability

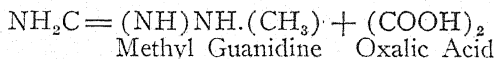
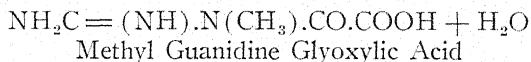
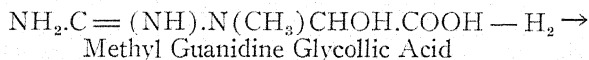
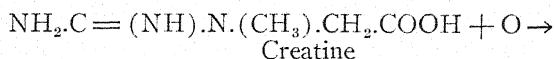
to split off ammonia in metabolism. The writer agrees with this view. It is evident that glycine and arginine form creatine and creatinine directly, and the other amino acids do so indirectly through the ammonia that may be liberated from them in metabolism. In many studies we have shown the great importance of ammonia in creatine formation, *in vivo* and *in vitro*.

The administration of creatine containing N^{15} resulted in the formation of creatine containing N^{15} bound to phosphate in muscle and internal organs (5). The rate of creatine formation in normal rats fed on a creatine-free diet was investigated with the aid of isotopes, N^{15} in both the sarcosine and amidine parts of creatine (6). From the decrease in the isotope concentration Bloch and Schoenheimer concluded that the amount of creatine synthesized daily corresponds to about 2 per cent of the total creatine of the animal's tissues. This amount was found to be excreted as creatinine daily. Within 29 days half of the creatine molecules in adult rats on a creatine-free diet were replaced by new creatine molecules. In contrast to the amidine moiety of arginine, the amidine part of creatine is not replaced in metabolism, showing that the C—C and C—N linkages remain intact. Creatine containing N^{15} was also not changed into urea or ammonia.

From past experience it is very difficult to accept the view that creatine does not undergo any metabolic transformations in the body besides its transformation into creatinine. It is well known that creatine is never quantitatively excreted when it is ingested and this amount of creatine, about 50 per cent (which is not excreted or changed into creatinine) is supposed to be stored or changed into some other compound. For instance, Kapeller-Adler and Toda (1) showed that methyl amine could be isolated from the urine after administration of creatine. The same occurred also in

all conditions associated with creatinuria (diabetes, pregnancy, muscular excretion, phosphorus poisoning, etc.).

Chanutin's (3) subject retained 55 gm. after the administration of massive doses of creatine. In the dogs studied by Benedict and Osterberg (1) and in the rabbits studied by Hahn and Fasold (1) administration of creatine caused over 50 per cent increases in muscle creatine. One cannot escape the conviction that some of this stored creatine was being changed into other substances. Schoenheimer's tracer technique probably does not give one the complete metabolic picture of what happens to this extra creatine in the body. It has long been known that the methyl group of creatine is stable and if Schoenheimer is correct in stating that the amidine group is also stable, it is still quite likely that creatine could be oxidized in the tissues to methyl guanidine and oxalic acid, which reaction has been shown by Baumann and Ingvaldsen (1) to occur *in vitro*. (The writer (37) has shown that creatine formed from creatinine in the presence of various tissues disappears from solution after about 10 days incubation.)



That this view is not improbable is shown by the fact that guanidine and its methyl and dimethyl derivatives have been shown to occur in normal urine (Kutcher and Lohmann (6), Findlay and Sharpe (1)). Major and Stephenson (1, 2), Sullivan, *et al.* (1), and Greenblatt (1) have also isolated

free guanidine from the urine of muscular dystrophy patients.

From their studies in creatine metabolism Schoenheimer and coworkers concluded that the main pathway for creatine formation was through glycine for the sarcosine part and arginine for the amidine group of glycoyamine, which is methylated to creatine by methionine.

In an addendum to their last article on the subject Bloch and Schoenheimer (7) take exception to the theory proposed by Beard and Pizzolato (11) whereby urea and glycine can condense to form glycoyamine, which can then be methylated by another molecule of glycine to creatine. It was stated that Beard and Pizzolato maintained that urea was one of the most potent creatine precursors. This is a misconception of our findings. It is clear that urea could not be as effective in creatine formation as glycine, glycoyamine or arginine, since it is rapidly excreted after ingestion. We believe that the precursors of urea would be better creatine formers than urea itself. It required 300 mg. of urea in our studies to give increases in creatine one or two days later. The injection of 100 or 200 mg. of urea gave no effect. But the injection of 100 mg., each, of urea and glycine, gave about the same increases as the injection of 300 mg. of urea alone. Beard and Pizzolato (11) stated in this connection, "It required 300 mg. of urea to give increases in creatine formation. This result was definite, convincing and reproducible, and shows the great importance of the size of the dose injected upon creatine formation."

In the negative studies by Bloch and Schoenheimer (7) only 23 mg. of urea nitrogen were *fed* daily for 3 days and no isotope could be found in the creatine isolated from the muscles. But in another study where 100 mg. per day of urea were fed, the creatine had a very small isotope content (0.014 atom per cent excess). They admit that the ammonia formed from the urea or other amino acids might have en-

tered the creatine molecule. We maintain that their dose was not large enough to give significant increases in the isotope of creatine. It would also be of interest if they were to study the effect of 100 mg. each of urea and glycine containing N^{15} fed together on creatine and creatinine formation.

The above criticism is very important. When an isotope is fed it mixes with the same non-isotope compound in the tissues, *i.e.*, glycine $N^{15} \rightarrow$ glycine, protein and glutathione, etc. This causes a dilution of the isotope marker and even though their analyses by the mass spectograph are very accurate, nevertheless, *sufficient concentrations of the isotope must be present to be determined* in the creatine isolated from the muscle or urine.

A serious objection to their theory that arginine and glycine are the only creatine precursors is that only 25 to 30 per cent of the creatine nitrogen arose from these two amino acids; all of the creatine nitrogen could not be accounted for by their tracer technique. The question might well be asked as to what is the origin and destination of this other 48 per cent of the creatine nitrogen. It is evident that more creatine was being formed than was accounted for in their technique. Another case in point is the fact that Schoenheimer and Rittenberg (14) showed that only a small amount of administered glycine containing N^{15} was used to form hippuric acid from benzoic acid, while the body proteins furnished by far the greater amount of glycine for this purpose. Griffith and Lewis (2), Probst and Londe (1), and Josephson (1), however, all showed that the excretion of hippuric acid was much higher when glycine was given with benzoic acid than it was when benzoic acid alone was given. Hence it is established, contrary to the findings of Schoenheimer, that exogenous or administered glycine is also used in the animal body to detoxicate benzoic acid.

Bloch and Schoenheimer (1, 7) have also shown that the

amidine group of creatine is formed from other amino acids besides arginine. They believe that the small amount of creatine formed from some other amino acids was through the ammonia that they yield in metabolism, yet when *immature rats* were used, "creatine samples with a moderate isotope could be obtained." It should be noted in this connection that we have, in most cases, used young, growing rats in our studies, while the New York investigators used for the most part adult animals. When they did, however, use immature animals, the creatine synthesized in these animals was 3 times as much as it was in the adult animals. If they obtained 0.014 atoms per cent in creatine from 100 mg. urea in adult animals, then, according to their statements, this should be 3 times greater, or 0.042 atoms per cent in young animals, and this value corresponds to 0.03 atoms per cent found in one of their studies with glycine and 0.021 atoms per cent found in another study with sarcosine. It should be remembered that it required 300 mg. of urea to give increases in creatine in rat muscle using the Jaffé reaction. If the amount of isotope in creatine is directly proportional to the amount of its precursor isotope fed, then the feeding of 300 mg. of urea should have given 0.042×3 or 0.126 atoms per cent in creatine, etc. This dose of urea may be considered unphysiological for the rat, but it does indicate that the precursors of urea may be potent creatine formers.

The statement was made that glycine and arginine were probably not the only creatine precursors, but that they were the chief ones. If it is assumed that a direct creatine formation takes place from arginine and glycine then our work shows that an indirect formation also occurs from many other substances. It is, therefore, evident that all of the creatine formed in Schoenheimer's studies was not accounted for by his tracer technique. For this reason the concentration of muscle creatine as determined by the Jaffé reaction

should also be made and compared to the results obtained by the tracer technique.

From their Table 1 (7) the following values were published:

TABLE 9

(After Bloch and Schoenheimer (7))

<i>Compound Administered</i>	<i>Isotope Content atom % excess</i>	<i>Total N Administered mg.</i>	<i>Isotope Content in Creatine and Creatinine atom % excess</i>	<i>Variation (from Lowest to Highest) per cent</i>
Glycine	4.5	23	0.030	
Glycine	4.5	25	0.078	160
Glycine	4.5	25	0.225	740
Sarcosine	4.5	23	0.021	
Sarcosine	4.5	23	0.055	160
Guanidine Acetic Acid	1.5	4.2	0.128	
Guanidine Acetic Acid	1.5	4.5	0.528	313

The per cent variations calculated by the author in the last column show that the tracer technique does not give reproducible quantitative results. It is possible that these variations are due to the difficulties in the isolation of muscle creatine or to differences in the amount of the same diet eaten by the animals. They might also be due to the fact that only a very few animals were used and creatine and creatinine contents of the muscles and urine are very variable under different experimental conditions. While it is appreciated that the New York investigator's work is painstaking and accurate, the data are rather limited and very few animals were used. While the positive evidence of the tracer technique for the transformation of one compound into another in the body cannot be denied, negative results by this technique might be due to any number of factors operating in the animal body at the time the tests were made. It is, therefore,

evident that, even though the results of Schoenheimer and coworkers have given us direct evidence for one mechanism of creatine and creatinine formation in the body, this fact should not detract from the value of the large mass of data obtained in the past using the Jaffé reaction for the determination of creatine and creatinine. Most of the facts of this type of metabolism proved by the New York investigators have long been known. For instance, arginine, glycine and guanidine acetic acid are very efficient creatine precursors (Arginine: Czernecki (1); Knopp (1); O. Neubauer (3); Thompson (1, 2); Crowdle and Sherwin (1); Gross and Steenbock (1); Hongo (1); Glycocyamine: Czernecki (1); Jaffé (1); Dorner (1); Thompson (1); Gibson and Martin (1); Baumann and Hines (2, 3); Palladin and Wallenberger (2); and Stuber, *et al.* (1)). Nevertheless the introduction of the tracer technique marks a definite advance in our methods of determining metabolic and other reactions in the body which would be impossible using other known techniques.

Bloch and Schoenheimer (7) have also recalculated the data given by Beard and Pizzolato (11) showing that most of our compounds have yielded more than 100 per cent of their total nitrogen for creatine formation. They stated that we showed that 10 mg. of creatinine injected into rats results in an increase in muscle creatine of 19 per cent, which is 520 per cent of the theoretical value. The inference is left that such reactions cannot possibly occur in the animal body.

It is evident that the New York investigators, in making the above calculations, have either not read our papers on the subject or they assume that chemical reactions occur in the animal body exactly as they do in a laboratory flask. Many metabolic reactions are taking place at all times. When glycine or other amino acids are ingested they may undergo several types of reaction and one of these may *stimulate* the

rate of another reaction. Confirmation of this view is found in the results of Hess and Sullivan (2) who showed that the ingestion of alanine, glycine, or glutamic acid increased the excretion of cystine. The results were stated to be due in a large measure to a stimulation of metabolism.

In several of our studies we have been impressed with the fact that more than the theoretical amount of creatine and creatinine can be obtained from their precursors. The smallest dose of creatinine administered gives the largest increases in muscle creatine. The question of creatinine retention is of much importance in this connection. In at least 6 studies the injection of only 4 mg. of caffeine caused increases of over 100 per cent in muscle creatine. At the same time there were increases in creatine and creatinine excretion of over 6,000 per cent. Again when an adult ingested 1 gm. of creatinine by mouth there may be from 10 to 20 gm. of extra creatine excreted into the urine. These results have been obtained in so many studies that we are unwilling to believe that they are due to experimental errors.

It is, therefore, evident that creatine and creatinine formation and excretion can be greatly stimulated under the conditions of our studies. It would seem, therefore, that the calculations of Bloch and Schoenheimer, while seemingly impossible from the chemical point of view, nevertheless are reasonable from the biochemical, physiological or pharmacological points of view. Further information in this connection will be found in Chapter VIII.

In their last publication on the subject Bloch and Schoenheimer (7) stated that we still held to our view that creatine was not transformed into creatinine, but that the reverse process takes place. They also stated that "this view is in disagreement with the results of all other investigators". One has only to read our publications and the monograph of Hunter (p. 132) to see that this is clearly a misstatement of the

facts. In fact, very few workers have accepted the creatine → creatinine transformation as *proved* in the body. Bloch and Schoenheimer referred to the criticisms of Fisher and Wilhelmi of our urea-glycine scheme for creatine formation. Fisher and Wilhelmi (1) also criticized our urea-glycine scheme and stated that our increases in muscle creatine were only apparent, as the massive doses of the amino acids were not absorbed and resulted in dehydration of the organs. How could dehydration of the organs occur if the amino acids were not absorbed? In fact, we *injected* the amino acids in this study which the English and German workers criticize. Finally, Bloch and Schoenheimer make this statement in regard to the work of Beard and Espenan on urea-glycocyamine synthesis, "However, the reaction product, which according to Lippich (1) is hydantoic acid, was not identified by isolation." In the studies of Lippich, urea and glycine were heated to 120° C.; in ours, the temperature never went above 80° C. Furthermore, when urea and sarcosine were heated together the Jaffé reaction obtained on the addition of alkaline picrate was destroyed by the creatinine enzyme of Miller and Dubois showing that creatinine alone was responsible for the reaction.

In conclusion, the technique used by Schoenheimer and coworkers differs greatly from ours. Therefore their criticisms of our results are unjustified. We maintain that they should also determine the creatine content of their rat muscles at the same time as they make their determinations by the mass spectograph. The Jaffé reaction will give the *total* creatine formed under the conditions of their experiment.

Almquist, *et al.* (1, 2) have shown that glycine is essential in the diet of the chick. In the absence of this amino acid muscular dystrophy develops. It was also shown that creatine increases the growth of the chick more than does glycine or acetates. In a personal communication to the writer, Dr.

Almquist stated that creatinine could likewise increase the growth of the chick. This is to be expected if creatinine is transformed into creatine (*Cf.* Chapter IX).

Hegsted, *et al.* (3) showed that arginine and glycine were necessary in the chick to form body tissue, creatine and feathers. Arginine and glycine together, but not alone, increased the muscle creatine content from 3 mg. per gm. to 4.2 mg. per gm. A marked growth response was shown in feathering leghorn hens. Arginine and glycine also prevented a typical paralysis which develops in chicks fed on their basal ration.

Further evidence of a stimulation of creatine formation is shown in the results of our purine studies (Beard and Pizzolato (15)).

TABLE 10

EFFECTS OF PARENTERAL INJECTION OF PURINE
DERIVATIVES UPON CREATINE FORMATION
(After Beard and Pizzolato (15))

Substance Injected	Number of Animals	Amount In- jected, mg.	Duration of Experi- ment, days	MUSCLE CREATINE			
				Maxi- mum, per cent	Mini- mum, per cent	Average, per cent	Increase, per cent
Controls	30			0.44	0.36	0.41	
Adenine	6	12	2	0.67	0.56	0.60	46
Guanine	3	2	2	0.72	0.72	0.72	75
Guanine	3	4	2	0.88	0.86	0.87	112
Guanine	3	6	2	0.93	0.87	0.89	117
Xanthine	6	6	2	0.78	0.58	0.65	58
Hypoxanthine	7	6	2	0.65	0.55	0.59	43
Uric Acid	12	6-12	2	0.66	0.51	0.59	43
Allantoin	8	6-12	2	0.67	0.58	0.63	53
Caffein	10	4-6	2	0.96	0.71	0.81	97
Theobromine	6	6	2	0.77	0.68	0.74	81
Theobromine	4	12	2	0.97	0.87	0.93	127

When 6 to 12 mg. quantities of xanthine, hypoxanthine, uric acid, or allantoin were injected the average increases in muscle creatine varied from 43 to 58 per cent above normal. Guanine, injected in doses from 2 to 6 mg., gave increases from 76 to 117 per cent above normal. Theobromine in doses

from 6 to 12 mg. gave increases from 81 to 127 per cent; and caffeine in doses from 4 to 6 mg. gave increases from 97 to 137 per cent. The increases in methyl groups from none in xanthine to two in theobromine and three in caffeine gave average increases, in muscle creatine, respectively, as follows: 58, 81, and 127.

Beard and Pizzolato (11) next studied the effect of injecting about 50 different compounds, alone and in various combinations, upon the formation of muscle creatine in the rat (Table 11). One hundred and sixty-six control and over 500 experimental animals were used. Each study was repeated three times before the results were accepted. These results were summarized on page 3 and will not be repeated here, except to state that the results indicate beyond question that the injected amino acids were either directly or indirectly transformed into muscle creatine in the rats.

TABLE 11

EFFECT OF PARENTERAL INJECTION OF AMINO ACIDS
AND RELATED SUBSTANCES UPON CREATINE
FORMATION (IN PER CENT)
(After Beard and Pizzolato (11))

Substance Injected	No. of Rats	Duration Amount of In- Experi- jected, ment, mg. days		MUSCLE CREATINE		
				Maxi- mum	Mini- mum	Average Increase
Controls	166			0.47	0.36	0.42
Glycine	6	100	1	0.70	0.46	0.55 30.9
Glycine	4	100	2	0.48	0.41	0.45 7.1
Glycine Anhydride	2	100	1	0.53	0.52	0.53 26.1
Glycine Anhydride	3	100	2	0.66	0.56	0.59 40.4
Glycine Anhydride	3	100	3	0.45	0.40	0.42 None
Glycyl-Glycine	3	100	1	0.77	0.73	0.76 80.9
Glycyl-Glycine	5	100	2	0.41	0.36	0.40 None
Di-Glycyl-Glycine	4	100	1	0.76	0.73	0.76 80.9
Di-Glycyl-Glycine	5	100	2	0.42	0.39	0.41 None
l-Leucyl-Glycine	4	100	1	0.74	0.66	0.70 66.6
l-Leucyl-Glycine	5	100	2	0.43	0.37	0.40 None
l-Leucyl-Glycyl-Glycine	3	100	1	0.75	0.75	0.75 75.0
l-Leucyl-Glycyl-Glycine	5	100	2	0.43	0.37	0.40 None
Sarcosine	6	100	1	0.60	0.48	0.54 28.5
Sarcosine	3	100	2	0.71	0.61	0.65 54.7
Sarcosine	4	100	3	0.60	0.50	0.55 30.9
Sarcosine	4	100	4	0.48	0.44	0.46 9.5

Origin of Creatine

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Substance Injected	Duration		Amount of				CREATINE
	No. of Rats	In- jected, mg.	Experi- ment, days	MUSCLE		Average	Increase
				Maxi- mum	Mini- mum		
Sarcosine Anhydride	3	100	1	0.61	0.51	0.55	30.9
Sarcosine Anhydride	5	100	2	0.83	0.57	0.70	66.6
Sarcosine Anhydride	4	100	3	0.41	0.37	0.40	None
Betaine-HCl	2	100	1	0.57	0.51	0.54	28.5
Betaine-HCl	8	100	2	0.69	0.58	0.63	50.0
Betaine-HCl	3	100	3	0.51	0.46	0.49	16.6
Betaine-HCl	3	100	4	0.50	0.48	0.49	16.6
Betaine-HCl	2	100	5	0.44	0.44	0.44	4.7
Betaine-HCl	3	5	1	0.43	0.38	0.41	None
Betaine-HCl	3	5	2	0.37	0.34	0.39	None
Betaine-HCl + Arginine	3	100	2	0.73	0.54	0.65	54.7
Betaine-HCl + Glycocyamine	3	100	2	0.56	0.52	0.54	28.5
Choline-HCl	3	5.0	1	0.50	0.45	0.47	11.9
Choline-HCl	3	5.0	2	0.54	0.44	0.50	19.0
Choline-HCl	4	5.0	3	0.50	0.48	0.49	16.6
Choline-HCl	4	5.0	4	0.52	0.42	0.46	9.5
Choline-HCl	2	7.0	2	0.59	0.58	0.59	40.4
Choline-HCl	2	8.7	2	0.62	0.60	0.61	45.2
Choline-HCl	2	10.5	2	0.74	0.72	0.73	73.8
Choline-HCl	2	12.2	2	0.66	0.55	0.60	42.8
Choline-HCl	2	15.0	2	0.64	0.63	0.64	52.3
Choline-HCl + Glycocyamine	3	5.0	2	0.64	0.63	0.64	52.3
Caffein	4	2	2	0.74	0.63	0.66	57.1
Caffein	10	4	2	1.16	0.85	1.00	138.1
Caffein	4	4	1	0.62	0.50	0.54	28.5
<i>D</i> -Methionine	2	100	1	0.39	0.39	0.39	None
<i>D</i> -Methionine	6	100	2	0.56	0.44	0.52	23.8
<i>N</i> -Methyl Methionine	2	100	2	0.86	0.86	0.86	104.7
Methyl Amine	10	50	2	1.06	0.57	0.64	52.3
Methyl Amine	2	100	2	0.79	0.77	0.78	85.7
Glycollic Acid	5	50-100	2	0.68	0.56	0.62	47.6
Glycocyamine	6	100	2	0.67	0.43	0.54	28.5
Glycocyamine + Leucine	5	100	2	0.53	0.43	0.46	9.5
Methyl Guanidine-HCl	3	5	2	0.37	0.34	0.37	None
Methyl Guanidine-HCl	3	10	2	0.45	0.34	0.40	None
Methyl Guanidine-HCl	3	15	2	0.62	0.50	0.54	28.5
Methyl Guanidine-HCl	3	20	2	0.68	0.60	0.64	52.3
Methyl Guanidine-HCl	4	25	2	0.54	0.47	0.51	11.4
Methyl Guanidine-HCl	4	35	Toxic to all animals				
Urea	4	100	1	0.44	0.41	0.42	None
Urea	4	100	2	0.40	0.33	0.35	None
Urea	4	200	1	0.38	0.36	0.37	None
Urea	4	200	2	0.37	0.32	0.35	None
Urea	2	300	1	0.59	0.49	0.54	28.5
Urea	10	300	2	0.96	0.60	0.76	80.9
Urea	4	300	3	0.42	0.37	0.40	None
Urea + Glycine	4	100	1	0.68	0.58	0.63	50.0
Urea + Glycine	11	100	2	0.82	0.60	0.69	64.2
Urea + Glycine	4	100	3	0.45	0.36	0.39	None

Substance Injected	No. of Rats	Amount In- jected, mg.	Duration of Experi- ment, days	MUSCLE		CREATINE	
				Maxi- mum	Mini- mum	Average	Increase
Urea +	4	100	1	0.85	0.81	0.83	97.6
Sarcosine		100					
Urea +	10	100	2	0.98	0.74	0.88	109.5
Sarcosine		100					
Urea +	4	100	3	0.45	0.39	0.42	None
Sarcosine		100					
Urea +	4	100	1	0.56	0.51	0.53	26.1
Betaine		100					
Urea +	7	100	2	1.03	0.56	0.84	100.0
Betaine		100					
Urea +	2	100	3	0.45	0.39	0.42	None
Betaine		100					
Urea +	4	100	1	0.59	0.54	0.56	33.3
Choline		5					
Urea +	7	100	2	0.85	0.62	0.75	78.5
Choline		5					
Urea +	4	100	3	0.44	0.37	0.39	None
Choline		5					
Urea +	4	100	1	0.60	0.52	0.56	33.3
Caffein		4					
Urea +	8	100	2	0.90	0.72	0.83	97.6
Caffein		4					
Urea +	4	100	3	0.47	0.45	0.46	9.5
Caffein		4					
<i>l</i> -Aspartic Acid	2	50	2	0.44	0.40	0.42	None
<i>l</i> -Aspartic Acid	4	100	2	0.53	0.47	0.51	21.5
<i>N</i> -Butyric Acid	4	25-50	2	0.44	0.40	0.42	None
<i>dl</i> -alpha-amino- <i>N</i> -Butyric	5	100	2	0.68	0.53	0.58	38.0
<i>dl</i> -alpha-amino							
Methyl Butyric	5	100	2	0.64	0.55	0.60	42.8
<i>N</i> -Caproic Acid	5	5.0	2	0.43	0.38	0.40	None
Methyl Caproate	3	12.5	2	0.40	0.39	0.40	None
Methyl Caproate	3	25.0	2	0.41	0.38	0.39	None
<i>dl</i> -alpha-amino- <i>N</i> -							
Caproic Acid	4	100	2	0.63	0.57	0.61	45.2
<i>dl</i> -alpha-amino							
Valeric Acid	6	100	2	0.67	0.59	0.64	52.3
<i>l</i> -Cystine	2	50	2	0.53	0.47	0.50	19.0
<i>l</i> -Cystine	2	75	2	0.57	0.51	0.54	28.5
<i>l</i> -Cystine	2	200*	2	0.50	0.48	0.49	16.6
Cysteine-HCl	6	100	2	0.61	0.52	0.55	30.9
Glutathione	2	33	2	0.59	0.58	0.59	40.4
Glutathione	3	66	2	0.69	0.61	0.65	54.7
Glutathione	4	99	2	0.69	0.57	0.62	47.6
Glutathione	2	125	2	0.67	0.58	0.63	50.0
Glutathione	3	150	2	0.68	0.53	0.63	50.0
Glycine Cysteine		33					
HCl Glutamic Acid	4	of each	2	0.70	0.54	0.60	42.8
Glutathione	3	50	1	0.63	0.60	0.61	45.2
Glutathione	6	50	2	0.72	0.63	0.67	59.5
Glutathione	3	50	3	0.50	0.48	0.49	16.6
Glutathione	4	50	4	0.53	0.49	0.51	11.4
Glutathione	4	50	5	0.46	0.39	0.42	None
<i>d</i> -Glutamic Acid	3	50	2	0.47	0.40	0.43	None

* This dose of cystine killed three animals.

Substance Injected	No. of Rats	Duration Amount of In-jected, mg.		Experi- ment, days	MUSCLE CREATINE			
					Maxi- mum	Mini- mum	Average	Increase
d-Glutamic Acid	4	100	2	0.52	0.47	0.50	19.0	
Glucose	4	100	1-2	0.42	0.36	0.38	None	
		200						
Hydantoin	2	50	2	0.81	0.71	0.76	80.9	
Hydantoin	2	100	2	0.87	0.80	0.83	97.6	
l-Leucine	8	100	2	0.55	0.46	0.48	14.2	
dl-Isoleucine	9	100	2	0.41	0.37	0.39	None	
dl-Lysine	2	100	1	0.36	0.36	0.36	None	
dl-Lysine	7	100	2	0.56	0.47	0.49	16.6	
Sodium Lactate	6	50-100	2	0.50	0.41	0.45	7.1	
Yeast Nucleic Acid	14	50-100	1-2	0.44	0.38	0.41	None	
d-Ornithine	3	50	1	0.45	0.40	0.43	None	
d-Ornithine	8	50	2	0.75	0.60	0.68	61.9	
d-Ornithine	3	50	3	0.48	0.44	0.46	9.5	
d-Ornithine	4	50	4	0.49	0.42	0.45	7.1	
d-Ornithine	6	100	2	0.78	0.59	0.68	61.9	
Sodium Pyruvate	5	50-100	2	0.44	0.39	0.41	None	
dl-Phenylalanine	9	100	2	0.41	0.36	0.41	None	
l-Phenylalanine	10	100	2	0.51	0.45	0.48	14.2	
l-Proline	3	50	2	0.42	0.34	0.37	None	
l-Proline	4	100	2	0.42	0.32	0.37	None	
l-Hydroxyproline	4	100	2	0.40	0.39	0.40	None	
l-Tryptophane	2	100	1	0.37	0.37	0.37	None	
l-Tryptophane	8	100	2	0.53	0.43	0.48	14.2	
di-Iodotyrosine	4	30	2	0.61	0.50	0.56	33.3	
di-Iodotyrosine	2	60	2	0.56	0.53	0.56	33.3	
di-Iodotyrosine	2	90	2	0.56	0.49	0.52	23.8	
dl-Valine	10	100	1-2	0.42	0.37	0.38	None	

The reader is no doubt bewildered by the large number of different substances that are able to increase creatine and creatinine formation and excretion after their administration. In considering the large number of metabolic changes which occur in the body in feeding and injection experiments, many direct and indirect effects on creatine and creatinine formation and excretion are possible from such substances which supply urea, arginine, creatinine or glycoamine for the amidine group of creatine and creatinine and glycine, sarcosine, methionine, etc., or other amino acids for the methyl group. The discovery of reactions of amination, deamination, transamination, transmethylation, and the "amidine shift", by Braunstein and Kritsmann (1), Schoenheimer, *et al.* (1-12), Borsook and Dubnoff (2, 3, 4), and du Vigneaud, *et al.* (1, 2), must be considered in this connection. Prac-

tically any amino acid, except proline or hydroxyproline, provided that it be fed or injected in sufficient amounts, can serve to increase creatine and creatinine formation and excretion.

Thomas (1) and Rose (3) expressed the view that exogenous arginine was not the mother substance of creatine and creatinine, but they believed that tissue arginine may be an intermediate in its formation. Our results with free arginine, however, were definite and convincing (Beard and Barnes (3), Beard and Boggess (28), Beard and Pizzolato (11). At this time (1928-30) we were convinced that the tissues make no distinction between free or combined amino acids in creatine formation. A recent statement by Rittenberg (15) is interesting in this connection, "When *l*-leucine was fed to rats, the arginine isolated from the proteins contained labeled nitrogen. Degradation showed that the N¹⁵ was mainly in the amidine group. This is the result of the urea cycle. As arginase is rather ineffective on arginine in the protein, this reaction probably took place while the amino acid was in the free state. If so, the arginine of the protein and the free arginine must rapidly interchange, as the N¹⁵ concentration of the amidine group is one half of that of the urinary urea."

Rose (3), in reviewing the evidence of Beard and Barnes (3) for the exogenous origin of creatine and creatinine, stated,

"We are thoroughly aware of the fact that the point of view indicated above is rejected in no uncertain terms by many students of creatine and creatinine, who prefer to regard creatine as one of the end products of exogenous protein catabolism. The problem is an exceedingly complex one involving a number of factors, such as variation in powers of retention, age and sex of the subjects, the carbohydrate supply, and probably many other as yet unrecognized influences. Under the circumstances, the reviewer cannot escape the conviction that the observed increases in creatine in the tissues or urine of normal adults follow-

ing excessive feeding of particular diets may be interpreted eventually in a satisfactory fashion without necessitating an acceptance of the doctrine of an almost unlimited creatine production from widely different exogenous sources. Perhaps a more satisfactory approach to the problem of the origin of an anabolic substance would be to limit the intake of a supposed precursor below the required level, rather than to add excessive quantities to a diet already carrying sufficient amounts to meet the demands of synthesis."

Beard and Boggess (4) considered this suggestion of Professor Rose. Normal adult rats were fed upon complete synthetic diets containing either 4 per cent casein or egg albumin as the sole protein for a period of 5 weeks. Re-feeding these animals on 25 per cent casein or egg albumin, or 21 per cent of glycine or glutamic acid for a period of 4 more weeks, caused average increases in the total creatine of the muscles varying from 20 to 56 per cent above that of some of the other animals fed on the 4 per cent protein diets. These results will assume more importance in connection with the theories of some of the myopathies discussed in Chapter XVII.

Beard and Pizzolato (11) submitted their urea-glycine scheme for creatine formation.* Fisher and Wilhelmi (1) offered several theoretical criticisms of this scheme, some of which are:

1. *Dehydration would increase muscle creatine.* But from their own published figures it is clearly seen that their urea-glycine animals actually had *less* total solids than their other two groups not injected with urea-glycine. In December 1935 Beard and Pizzolato (11) obtained data on this point. The control group of rats ate the stock diet only; the experimental

* The utilization of urea and similar nitrogenous compounds in place of protein is very favorable in several species of animals (Auer (1); Lilien-cron (1); Sauer (1); Hart, *et al.* (1); Harris and Mitchell (3, 4)). Concentrated foodstuffs containing ammonium salts, glycine, etc., are also being produced at the present time.

group had 100 mg. each of urea and glycine injected once. Both groups had access to water. There were 25 per cent total solids in the muscles of each group and 70 per cent increase in creatine formation in favor of the urea-glycine group over that in the control group. These results were so obvious to us that we did not consider it necessary to publish them at the time.

2. *Muscle sampling.* The claim was made that our method of muscle sampling was incorrect as different leg muscles of the rat contain different amounts of muscle creatine. The author found the following values for the creatine content in mg./100 gm. in the following rat muscles: biceps femoris, 406; gluteus maximus, 450; gastrocnemius, 465; soleus, 450; vastus lateralis, 470; semitendinosus, 465; semimembranosus, 430; and caudofemoralis, 446. The average value was 446 mg./100 gm. Baker and Miller (1) found a mean value of 540 mg. creatine, 96 per cent of which was true creatine. Chanutin (1) reported a value of 449 mg./100 gm. in rat muscle, and our average control values for 500 young rats fed on our stock diet was 420 ± 2 mg./100 gm. In contrast to these figures Fisher and Wilhelmi reported a mean value of only 356 mg./100 gm. for the gastrocnemius. From their Table 1 the value of 299 to 305 mg./100 gm. is also reported for this same muscle and the values in Table 2 run from 316 to 339 mg./100 gm. It is, therefore, possible that the technique used in the determination of muscle creatine is inaccurate and this casts considerable doubt upon the validity of their published figures for muscle creatine.

Fisher and Wilhelmi stated that only a 6 per cent increase in muscle creatine was possible from consideration of statistical data, yet their highest value for muscle creatine was 33 per cent above that of their lowest value. It is also known from the work of Chanutin (3), Benedict and Osterberg (1), Hahn and Fasold (1), and our own that, after creatine ingestion, the concentration of muscle creatine is raised from

10 to 50 per cent above normal. This shows the large capacity of the muscle to store creatine.

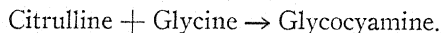
3. *Creatine synthesis.* Fisher and Wilhelmi stated that increases in creatine formation and in creatine excretion could not be accepted as showing an increase or synthesis of creatine in the tissues. Reference to the results of Bloch and Schoenheimer in Table 9 shows the inaccuracy of this statement.

4. *Stimulation of creatine formation and excretion.* They claimed that Beard and Pizzolato were attempting to show a *synthesis* of creatine when our values were much higher than required by theory. In many of our studies we have never claimed that we obtained only a synthesis, but we did claim that under these conditions there was a *stimulation* of creatine formation and excretion (*Cf.* reply to these same criticisms of Schoenheimer, *et al.* above, p. 64). On page 140 of their paper Fisher and Wilhelmi made this statement, "That the dual response (creatine formation and excretion) to the same substance does not increase the probability of creatine synthesis having occurred is conclusively shown in the work on rats, (Chanutin (2)); guinea pigs, (Palladin and Kudrjawzewa (1)); rabbits, (Myers and Fine (1)); and in Rose's review of the subject (3)." This is a misreading of some of these papers since Chanutin, Palladin and Myers were not concerned with the synthesis of creatine at all. Because the creatine content of the muscles may be increased in *starvation*, Fisher and Wilhelmi concluded that creatine formation and excretion during *amino acid feeding* is not a true criterion of creatine synthesis. It is only when a quantitative increase in creatine is followed by a quantitative decrease in its precursor in *in vitro* studies that they are willing to concede a synthesis of creatine. Since a great many metabolic reactions are reversible in the body, it is impossible to reach this criterion under experimental conditions. Also, to accept it, one must not believe that any substance in the body can

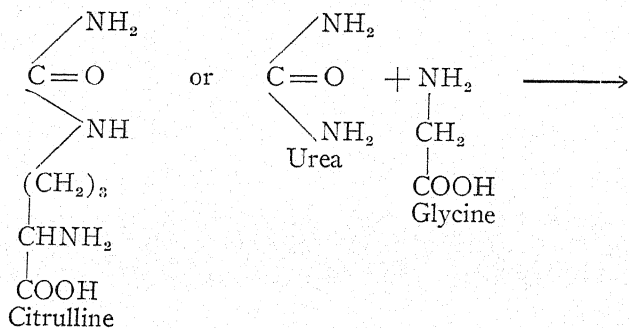
be formed at all unless a quantitative decrease in its precursor can be determined. Even though they showed a quantitative transformation of arginine into creatine in their heart perfusion studies, it is well known that this amino acid is transformed not only into creatine, but also into ornithine and urea and possibly other substances in the tissues. Hence no one believes that all of the arginine produced in the body is quantitatively transformed into creatine. Also the results of *in vitro* studies furnish only presumptive evidence of what may occur in the body.

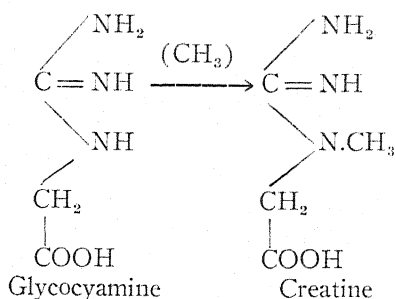
From the results of recent studies, the increase in creatine formation in starvation, if it occurs, is due to a synthesis from the tissue amino acids, and the creatinuria represents the creatine liberated from the muscles and excreted into the urine. It can be stated that their article is filled with theoretical criticisms of our work and none of these criticisms are acceptable to Beard and Pizzolato (Beard (32)).

Further support for our theory that urea (or its precursors) take part in creatine synthesis is furnished by the results of recent studies by Borsook and Dubnoff (4) who showed that the following reaction takes place *in vitro* in the presence of rat kidney tissue:



Creatine formation from citrulline, or urea, with glycine may be written:





Since Beard and Pizzolato (11) showed that the amino group of the amino acid was essential for creatine formation it is possible that the above compounds add ammonia, the NH group replacing the O attached to the guanidine C atom, to form the guanidine group of glycocyamine or creatine. Furthermore, additional evidence for this type of reaction occurring in the body is shown in Krebs and Henseleit's (1) well known scheme for urea formation from ornithine, citrulline, and arginine, as follows:

1. $\text{NH}_3 + \text{H}_2\text{CO}_3 \rightarrow \text{NH}_2\text{CO.OH} + \text{H}_2\text{O}$
Carbamic acid
2. $\text{NH}_2\text{CO.OH} + \text{NH}_2(\text{CH}_2)_3\text{CHNH}_2\text{COOH} \rightarrow$
Carbamic acid Ornithine
 $\text{NH}_2\text{CO.NH.}(\text{CH}_2)_3\text{CHNH}_2\text{COOH} + \text{H}_2\text{O}$
Citrulline
3. $\text{NH}_2\text{CO.NH.}(\text{CH}_2)_3\text{CHNH}_2\text{COOH} + \text{NH}_3 \rightarrow$
Citrulline
 $\text{NH}_2\text{C.}(\text{NH}).\text{NH}(\text{CH}_2)_3\text{CHNH}_2\text{COOH} + \text{H}_2\text{O}$
Arginine
4. $\text{NH}_2\text{C}(\text{NH}).\text{NH.}(\text{CH}_2)_3\text{CHNH}_2\text{COOH} + \text{H}_2\text{O} +$
Arginine
Arginase $\rightarrow \text{NH}_2\text{CO.NH}_2 + \text{NH}_2(\text{CH}_2)_3\text{CHNH}_2\text{COOH}$
Urea Ornithine

It was also shown in the author's laboratory that arginine and ornithine (11) and ammonium carbonate (38) were creatine formers (Tables 11 and 12).

TABLE 12

EFFECT OF INCUBATION OF AMMONIUM CARBONATE AND SARCOSINE AT 37° C. WITH AND WITHOUT PRESENCE OF VARIOUS TISSUES ON CREATINE AND CREATININE FORMATION
(Beard, unpublished observations)

Tissues Added to Solutions	DAYS OF INCUBATION AT 37° C.							
	5		10		15		20	
	P.C.*	C. as C.**	P.C.	C. as C.	P.C.	C. as C.	P.C.	C. as C.
(mg. per 100 cc. solution)								
Control	0.9	0.0	1.3	0.7	1.0	0.1	1.0	0.0
"	0.9	0.1	1.1	0.4	0.9	0.0	1.0	0.0
"	1.0	0.2	1.0	0.5	1.0	0.3	1.1	0.0
Liver, 4 gm.	0.8	0.8	1.2	0.4	1.1	0.5	1.0	0.4
" " "	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Muscle, 4 gm.	0.9	4.9	1.5	6.1	1.7	5.8	1.8	5.4
" " "	3.0	5.0	1.1	2.2	0.0	0.0	0.0	0.0
Intestine, 4 gm.	0.9	0.9	1.3	0.7	1.1	0.6	1.0	0.9
" " "	1.3	2.6	0.0	0.0	0.0	0.0	0.0	0.0
Kidney, 4 gm.	0.9	0.1	1.1	0.4	0.9	0.4	1.0	0.0
" " "	3.2	2.0	0.0	0.0	0.0	0.0	0.0	0.0
Lung, 4 gm.	0.7	0.0	1.0	0.2	0.9	0.4	1.0	0.0
" " "	2.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Heart, 2 gm.	0.8	0.4	1.1	0.7	1.0	0.2	1.0	0.9
" " "	0.7	0.9	0.0	0.0	0.0	0.0	0.0	0.0

EFFECT OF AUTOCLAVING FOR 4 HOURS AT 15 LB. PRESSURE AFTER INCUBATING AT 37° C. FOR 20 DAYS

	P.C.*	C. as C.**	P.C.	C. as C.	P.C.	C. as C.
Methyl urea + Glycine	(1)3.2	25.5	(2)2.9	26.3	(3)3.8	23.3

EFFECT OF HEATING SOLUTIONS AT 60° C. FOR 4 DAYS

Methyl urea +

Glycine (4)17.0 17.0 (5)18.7 16.6 (6)16.6 15.7

* Preformed Creatinine.

** Creatine as Creatinine.

Studies on the *in vitro* formation of glycoxyamine, creatine and creatinine have also been carried out by a number of workers, as follows:

- (1) Glycoxyamine \rightarrow Creatine (Borsook and Dubnoff (2, 3)).
- (2) Arginine + Glycine \rightarrow Glycoxyamine (Borsook and Dubnoff (4)).
- (3) Citrulline + Glycine \rightarrow Glycoxyamine (Borsook and Dubnoff (4)).
- (4) Arginine + Glycine (in man) \rightarrow Glycoxyamine (Borsook, Dubnoff, Lilly and Marriott (5)).
- (5) Gelatin \rightarrow Glycoxyamine (Borsook, Dubnoff, Lilly and Marriott (5)).
- (6) Isolated rat tissues \rightarrow Creatine (Baker and Miller (2)).
- (7) Arginine + Glycine + Thyroxin \rightarrow Creatine (Shibuya (1)).
- (8) Glycine + Glycoxyamine \rightarrow Creatine (Shibuya (2)).
- (9) Urea + Glycine \rightarrow Glycoxyamidine (Sasaki (1)).
- (10) Urea + Glycine \rightarrow Glycoxyamine (Beard and Espenan (21)).
- (11) Sarcosine + Urea \rightarrow Creatine (Beard and Espenan (21)).
- (12) Glycine + Cyanamide \rightarrow Glycoxyamine (Beard and Espenan (21)).
- (13) Sarcosine + Cyanamide \rightarrow Creatine (Beard and Espenan (21)).

- (14) Glycocyamine + Glycine + Heart tissue \rightarrow Creatine (Bach (1)).
 - (15) Arginine — (Heart perfusion) \rightarrow Creatine (Fisher and Wilhelmi (2)).
 - (16) Glycocyamine + Glycollic acid \rightarrow (Heart perfusion) \rightarrow Creatine (Davenport, Fisher and Wilhelmi (3)).
 - (17) Ammonium Carbonate + Sarcosine + muscle tissue \rightarrow Creatine and Creatinine (Beard (38)).
 - (18) Methyl Urea + Glycine \rightarrow Creatine and Creatinine (Beard (38)).
- (The addition of a few drops of concentrated ammonia greatly increased the speed of reactions 13 and 18.)

Beard and Espenan (21) studied *in vitro* the synthesis of glycocyamine from urea and glycine, and from glycine and cyanamide, and of creatine from sarcosine and urea, and sarcosine and cyanamide. Evidence was obtained that muscle tissue contains an enzyme which hastens these syntheses. This finding was confirmed in an ammonium carbonate-sarcosine study (38). This enzyme did not occur in liver and kidney tissue. The presence of the muscle enzyme, however, is not necessary if the temperature of the reaction is increased or if larger concentrations of the reacting substances are employed or if a longer time is allowed for the reactions to occur. It was found that the addition of a few drops of ammonia greatly increased the speed of some of these reactions (Cyanamide + Glycine), (Cyanamide + Sarcosine), and (Methyl Urea + Glycine). Glycocyamine and creatine were isolated and identified as products of the reaction. The yield of creatine (and creatinine) was 86 per cent of the theoretical amount that could be formed from sarcosine. These synthetic substances, when injected into rats, caused increases in muscle creatine varying from 20 to 45 per cent

above normal. These studies were controlled by using the specific creatinine enzyme of Miller and Dubos (1, 2, 3).

The author does not believe that these results are in conflict with those of Borsook and Dubnoff discussed above. It is erroneous to believe that there is only one mechanism of glycoyamine and creatine formation or that there is any one tissue, to the exclusion of the others, where synthesis and methylation occur. Creatine can also be formed *in vitro* from creatinine in the presence of different tissues without regard to questions of synthesis of glycoyamine or methylation of glycoyamine to creatine (Beard (37)). This is simply a hydration of creatinine to creatine (see Chapters IX and X).

Fitzgerald and Schmidt (3) showed many years ago that bacteria could synthesize creatinine, and Sears (1, 2) reported that creatinine could be formed in considerable quantities from peptone by *Proteus vulgaris*. Fish and Beckwith (1) also observed that creatinine was formed from several bacterial species. Their results for the amino acids are listed in Table 13.

TABLE 13

PRODUCTION OF CREATININE BY PROTEUS
FROM A SERIES OF AMINO ACIDS IN THE
PRESENCE OF GLUCOSE
(After Fish and Beckwith (1)).

(0.1 per cent solution of the amino acids)
(Results expressed in mg./100 cc. of solution)

<i>Amino Acid</i>	<i>3 days</i>	<i>6 days</i>
Glycine	4.17	4.0
<i>dl</i> -Alanine	3.46	2.0
<i>dl</i> -Glutamic Acid	2.50	1.5
Cystine	3.90	4.6
Phenylalanine	2.80	2.0
<i>dl</i> -Leucine	2.50	2.3
<i>dl</i> -Tyrosine	2.80	3.5
<i>dl</i> -Aspartic Acid	2.10	3.9
<i>d</i> -Arginine HCl	2.30	2.0

In their Table 3 it was shown that glycine or urea alone did not form much creatinine. When glycine, urea and glucose were used together there was formed 13.3 mg. of creatinine in 7 days by *Proteus*. Arginine was not as effective as glycine for the production of creatinine. Ammonium sulfate could not replace urea in creatinine synthesis with glycine. In the case of both glycine or arginine, urea and glucose had to be present for the best formation of creatinine. The destruction of the guanidine portion of arginine with arginase activated with manganese, still caused the formation of creatinine. This is additional evidence for the participation of urea in the synthesis of creatinine by bacteria.

The following reactions have been proposed by various groups of workers to show the possible formation of creatine and creatinine in the body:

1. Urea + Glycine \rightarrow Glycocyamine + Glycollic acid \rightarrow Creatine (Beard and Pizzolato (11); Kelly and Beard (14)). (Methyl urea can replace urea in this reaction.)

2. Urea + Glycine \rightarrow Glycocyamidine + Glycollic acid \rightarrow Creatine (Beard and Pizzolato (11); Kelly and Beard (14)).

3. Arginine \rightarrow Glycocyamine + Glycollic acid \rightarrow Creatine (Tripoli and Beard (5); Davenport, *et al.* (3)).

4. Arginine + Glycine \rightarrow Glycocyamine + Methionine \rightarrow Creatine (Bloch, *et al.* (6, 7); Borsook and Dubnoff (2, 3); du Vigneaud, *et al.* 1, 2); Jukes and Almquist (4)).

(This is considered as *the* biological reaction for the synthesis of creatine by these investigators, the glycine being obtained from proteins, the amidine group from arginine, and the methyl group from methionine.)

5. Sarcosine \rightarrow Glycine + Arginine \rightarrow Glycocyamine \rightarrow Creatine (Beard and Pizzolato (11)).

6. Sarcosine $\xrightarrow{-\text{CH}_3}$ Glycine + Arginine \rightarrow Glycocyamine

mine \rightarrow Creatine (Bloch, *et al.* (7); Borsook and Dubnoff (4)).

7. Glycine \rightarrow Glycollic Acid + Glycocyamine \rightarrow Creatine (Bach (1)).

8. Glycine + CO_2 + $2\text{NH}_3 \rightarrow$ Glycocyamine \rightarrow Creatine (Harrow (1)). (In unpublished studies the author has shown that this reaction occurs *in vitro*.)

9. Glycine + Carbamic Acid \rightarrow Hydantoic Acid + $\text{NH}_3 \rightarrow$ Glycocyamine \rightarrow Creatine (Mathews (1)).
+ H_2O

10. Creatinine $\xrightarrow{\quad\quad\quad}$ Creatine (Beard and Jacob (17), Beard (37), and Jukes and Almquist (4)).

Added interest is attached to reactions 8 and 9 since Ritzenberg and Waelsch (13) and Evans and Slotin (1) have shown that about half of the carbon of urea is derived from carbon dioxide in the body. It is also well known that carbon dioxide, ammonia, and ornithine react to form citrulline in Krebs and Henseleits' scheme for arginine and urea synthesis in the body.

Since it has been shown by the author that urea can take part in creatine synthesis in the body it is evident that the precursors of urea should also take part in this process. In our laboratory it was shown in 1938 that arginine, ornithine and urea can all take part in creatine synthesis (11). Since urea can also be formed from ammonia and carbon dioxide in the body, it is likely that these substances also take part in the synthesis. Beard and Barnes (3) and Beard and Pizzolato (11) showed that the amino group of the amino acid was necessary for creatine formation and Bloch and Schoenheimer (1, 2) showed that ammonia containing N^{15} could be transformed into creatine containing N^{15} . Beard (38) incubated ammonium carbonate with sarcosine at 37°C ., with and without the addition of different tissues and heated

methyl urea and glycine together at 60° C. The addition of ammonia increased creatine formation here by 1,200 per cent. The results obtained are given in Table 12.

Even though none of the evidence presented in this chapter indicates how creatine and creatinine are formed under normal conditions of metabolism, nevertheless the above experimental evidence leaves no doubt that these substances are formed during protein metabolism from the catabolism of the amino acids.

CHAPTER VII

METHYLATION OF GLYCOCYAMINE TO CREATINE. RELATION OF METHYL- ATION PROCESS TO STRUCTURE OF THE LIVER AND KIDNEYS. SITE OF CREATINE, CREATININE AND GLYCOCYAMINE FORMATION IN THE BODY

BEFORE DISCUSSING the mechanism of the methylation of glycocyamine to creatine let us consider a few types of methyl compounds that are of biological interest. These are:

1. CH_3 groups attached to N as in choline, the betaines, etc., the methyl groups being easily split off. The methyl group of creatine is stable.

2. CH_3 groups attached to a carbon in a ring, thus skatole — $\text{CH}_3 \rightarrow$ indole, etc., by hydrolysis.

3. CH_3 groups attached to most of the straight and branched chain amino acids, as in alanine, leucine, etc., the methyl group being split off by hydrolysis.

4. CH_3 groups attached to the S, as in methionine, the methyl group being split off by hydrolysis to homocystine.

5. CH_3 groups attached to some of the amino acids, such as methyl glycine, methyl methionine, methyl alanine, and methyl leucine (also methyl urea).

Handler, *et al.* (1) have shown that broken cell suspensions of rat kidney and liver oxidatively demethylate the

N-methyl derivatives of *dl*-methionine, *dl*-alanine, and *dl*-leucine. This is due to the *d*-amino oxidase. Handler, *et al.* (2) also showed that liver demethylates sarcosine to glycine. Thus, it would seem that hydrolytic and oxidative reactions are involved in the above types of reactions.

Much interest has recently been shown in the nature of the methylating agent in creatine formation from glyco-cyamine. Stekol and Schmidt (1) were the first to show that *dl*-methionine increased creatinine excretion in the dog. Beard and Pizzolato (11) showed that the injection of methionine increased creatine formation in young rats about 25 per cent (which we have found to be the equivalent creatine formation from 1 methyl group), while methyl methionine increased it over 100 per cent. Kelly and Beard (14) observed similar increases in creatine excretion after the injection of methyl urea as compared to urea.

These results are probably the first to establish the reaction of "transmethylation" in the body. In the formation of creatine the methyl group must be transferred to glyco-cyamine from some other compound, and the writer has shown that there are a number of compounds that can act as methyl donors (*Cf.* Kelly and Beard (14) below).

Borsook and Dubnoff (2, 3, 4, 5) showed that glyco-cyamine could be methylated to creatine by methionine in the presence of liver slices from various animals. Creatine formation from arginine and glycine was rapid in the presence of kidney slices. Arginine and sarcosine yielded glyco-cyamine and not creatine. This indicated, as has been shown by other workers (Bloch and Schoenheimer (4); Handler, *et al.* (2)), that sarcosine is demethylated without deamination to glycine in creatine synthesis. Du Vigneaud, *et al.* (1, 2) next marked the methyl group of methionine with deuterium and definite evidence of the shift of this group to creatine and choline was obtained. Fifty-six per cent of the methyl groups

of choline and 69 per cent of those of creatine were derived from the dietary methionine. Choline and betaine may also yield their methyl groups for creatine formation since the feeding of these compounds with deuterium in their methyl groups resulted in the excretion of deuteriocreatinine (du Vigneaud, *et al.* (2, 3)). Of much further interest in this connection is the finding of Griffith and Wade (1, 3) that choline deficiency results in hemorrhagic degeneration in the kidneys of young rats. This is a symptom of methyl deficiency which is prevented by choline, betaine and methionine. Schoenheimer, Borsook and du Vigneaud and their associates consider that the biological synthesis of creatine occurs *only* through glycine and arginine to form glycocyanine (in the kidney (Borsook)) which is then methylated to creatine in the liver (Borsook).

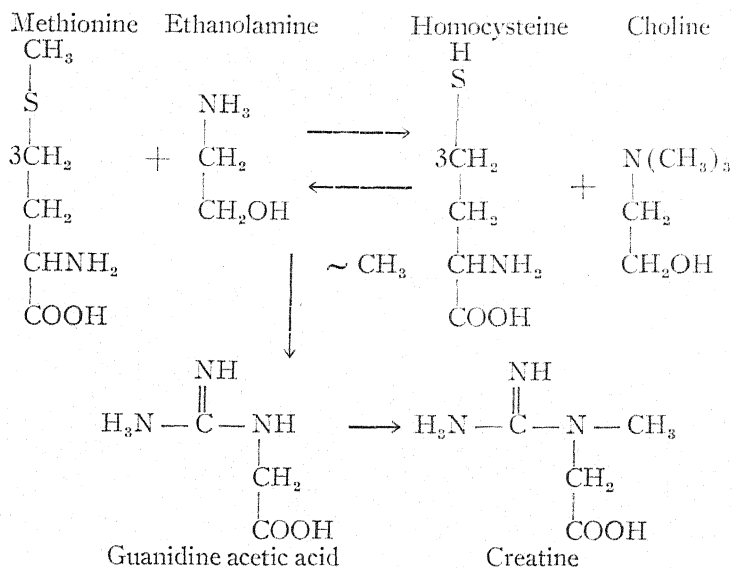
Borek and Waelsch (1) stated that the methylation of glycocyanine to creatine required only about 2 per cent of the added methionine. This corresponds approximately to the amount of sulfate formed from methionine in their experiments with liver slices. The sulfur of methionine is probably oxidized to sulfate if the methyl group is accepted by another compound.

Jukes and Almquist (4) suggested that creatine was formed in the chick from arginine and glycine and that methionine was the methylating agent in this connection. They, however, offered no experimental proof of this, and stated, "In fact a methionine deficient diet which will not support growth does not appreciably inhibit the formation of muscle creatine in the chick." The muscular weakness and deficient feather formation produced in chicks by a glycine deficient diet is completely cured by feeding creatine (Almquist and Mecchi (2), Almquist, *et al.* (3), Hegstead, *et al.* (3) and Jukes (5). Arginine does likewise (Hegstead, *et al.* (3)).

The methylation theory of the New York and California workers discussed above is too specific. If the view be accepted that sarcosine is only demethylated into glycine in creatine formation, then it must be assumed that equivalent amounts of sarcosine and glycine would give the same increases in creatine formation and excretion. Schoenheimer showed that the amount of glycine formed from sarcosine was about the same as if glycine alone had been administered. This is clear, but the *methyl group* of sarcosine is not accounted for in their studies. In our studies sarcosine has always given *more* creatine formation and excretion than did equivalent amounts of glycine. This increase, above that obtained from glycine alone, is equivalent to about 20 per cent *which is due to the methyl group of sarcosine* (Beard and Pizzolato (11); Beard (13); Kelly and Beard (14); Beard and Pizzolato (15); Koven and Beard (16)). It is, therefore, established that the methyl group and the glycine moiety of sarcosine both take part in creatine synthesis when sarcosine is administered (Cf. Chapter VIII). The writer has recently observed an increase of 1,200 per cent in creatine formation when a few drops of ammonia are added to methyl urea and glycine heated to 60° C.

The view that glycine, arginine, glycocyamine, and methionine are the only amino acids that take part in creatine synthesis is also too specific. They may be the most important ones, but there are other substances, as the following discussion will show, that increase creatine and creatinine formation and excretion in the body.

From the work of du Vigneaud, *et al.* (2) it would appear that the methyl group must be supplied to the body in the form of a methyl donator. The methyl of creatine is stable and cannot furnish methyl to the body. The following relation exists between the compounds reacting in the methylation of glycocyamine to creatine,



Methionine and choline, using Lipmann's terminology, contain the methyl group with a very high potential, designated by $\sim \text{CH}_3$. (The writer is convinced that the same is true of the methyl groups of many other compounds, especially caffeine and methyl methionine.) From these compounds distribution takes place with a variety of transfer enzymes to reversible or irreversible acceptors. A large part of the methyl group is needed for creatine synthesis. It was postulated that the N-methyl group was oxidized to an N-methylol group which dissociates, yielding a mole each of glycine and formaldehyde. (Methyl alcohol was not formed in place of formaldehyde).

Kelly and Beard (14) showed that methylating agents, such as methyl alcohol, methylamines, glycine, glycolic acid and paraformaldehyde, each increased creatine formation and excretion (Table 14). Since sarcosine gives an increase in creatine formation above that obtained from similar amounts

TABLE 14

CREATINE AS A PRODUCT OF METHYLATION *
(After Kelly and Beard (14))

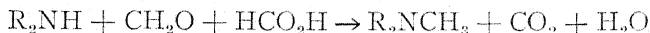
Substance Injected	No. of Rats	Average Muscle Creatine, per cent	Substance Injected	Urine Creatine (Total Extra Creatine Excreted in 8 Days),	
				No. of Rats	mg.
Methylamine	2	0.58	Methyl Urea	2	120
Dimethylamine	9	0.46-0.50	Methyl Alcohol	4	25
Trimethylamine	9	0.46-0.50	Methylamine	2	85
Methyl urea	18	0.46-0.60	Dimethylamine	4	101
Methyl alcohol	2	0.68	Trimethylamine	2	48
Glycollic acid	2	0.55	Acetylcholine	2	12
Paraformaldehyde	3	0.57	Acetylcholine	2	0
Methyl Iodide	2	0.53	Mecholyl	2	58
Acetyl Choline	2	0.43	Prostigmin	2	0
Prostigmin	4	0.56	Glycollic Acid	2	53
Acetylcholine + Prostigmin	3	0.66	Paraformaldehyde	3	32
Mecholyl	2	0.50	Methyl Iodide	2	54
Methyl Urea	2	0.65			
Methyl Alcohol	4	0.56-0.68			

* The average muscle creatine of 87 control animals in this study was 0.42 per cent.

of glycine (equivalent to that obtained from 1 methyl group) and since Handler, *et al.* have shown that 1 mole of formaldehyde is formed from sarcosine when it is demethylated to glycine, it is possible that formaldehyde may be formed in the tissues under these conditions and serve as a methylating agent. Formaldehyde can also be oxidized in the body to formic acid which is excreted as sodium formate (Sollmann (1)). The presence of small amounts of formic acid in the body has been suggested by Orskov (1) and in urine by Dakin, *et al.* (1). Fosse and de Larambergue (1, 2) showed that cyanamide is formed *in vitro* by the oxidation of sugars in the presence of ammonia and that formaldehyde is an intermediate product of the reaction. Of particular interest

in this connection are the observations of Schweitzer (1) who showed that the tyrosinase of potatoes would oxidize glycine to form formaldehyde, carbon dioxide and ammonia in the presence of alkali. Since glycine increases creatine and creatinine formation in the body it is seen that formaldehyde may possibly be an intermediate product in the methylation of glyco-cyanine to creatine.

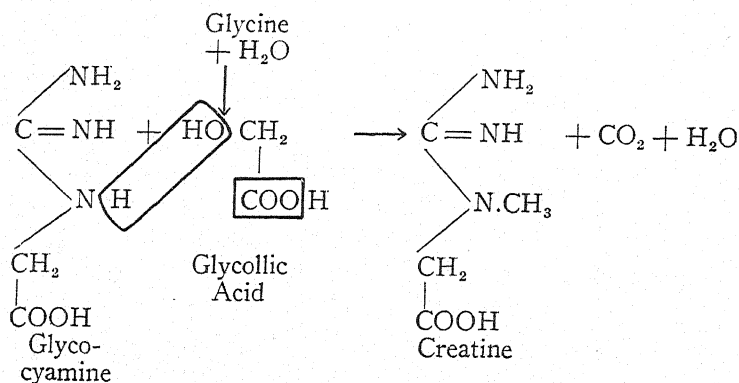
Sommelet and Farrand (1) prepared trimethylamine from ammonium formate, formaldehyde and formic acid. The reaction would be as follows:



The same type of reaction occurs with the amino acids and it would seem that the main methylation reaction would consist of the formation and subsequent reduction of a methylol derivative by the formic acid. Clarke, *et al.* (1) showed that amino acids react with formaldehyde in formic or acetic acid solution with detachment of some of the nitrogen in the form of volatile bases. Dimethyl derivatives were isolated from the reaction in formic acid with glycine and other amino acids. Formaldehyde condenses with urea to give methylol urea ($NH.CONHCH_2OH$) (Whitmore (1)).

Hoppe-Seyler (1) showed that glycine could act as a methylating agent. Challenger (1) pointed out that the biological methylation in microorganisms, plants, and animals may take place through the action of this amino acid. Through its deamination products, glyoxylic acid and formaldehyde, it is capable of methylating other amino acids, including itself, to yield sarcosine and betaine. Tripoli and Beard (5) suggested that glycine, as well as other amino acids, could supply the methyl group necessary for creatine formation. The same views were expressed by Mourot (1) and Bach (1). Kelly and Beard (14) showed that compounds containing methyl groups, which, however, are known

not to be methylating agents, do not influence creatine synthesis. Those compounds containing methyl groups *attached to nitrogen*, such as is found in the methyl amines, methyl urea, mecholyl (acetyl-beta-methylcholine chloride), caffeine and other purines and related substances, increased creatine formation and excretion. Glycine by hydrolytic deamination, may be converted into glycollic acid, which may then act as the methylating agent in creatine formation. Davenport, *et al.* (3) have shown that glycoeyamine and glycollic acid increase the total creatinine content of isolated perfused rabbit hearts and that glycollic acid acts as the methylating agent in this connection. Milhorat and Toscani (1) observed that glycollic acid caused a slight increase in creatine excretion in some of their myopathy patients. Beard (9) showed that the injection of arginine or glycollic acid in the hepatectomized dog caused an increase in creatine formation in the muscles 8 hours after the injection. From these results it would also appear that glycine may act as a methylating agent through glycollic acid in the body. The reaction may be written as follows:

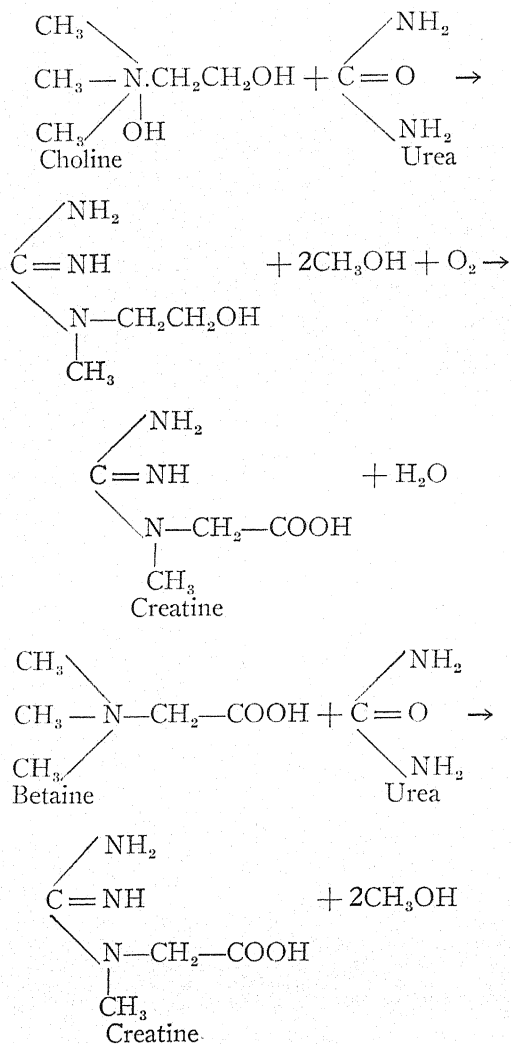


The purines and their methylated derivatives also play a very important rôle in this methylating process. It is estab-

lished from the earlier work of Abderhalden and Buadze (2, 3, 4), Chrometzka (1) and Zwarenstein (1) that purines, methylated purines, nuclear material, uric acid, hydantoin and methyl hydantoin are precursors of creatine. Beard and Pizzolato (15) and Koven and Beard (16) showed that creatine formation in the muscles and excretion in the urine were directly proportional to the number of methyl groups up to three present in the supplement injected (from xanthine, 59 per cent to dimethyl xanthine, theophyllin, 80 per cent to trimethyl xanthine, caffein, 99 per cent). One methyl group, therefore, again produced an increase in creatine formation above normal equivalent to about 20 per cent. Other purines and uric acid also increased creatine formation and excretion.

The effect of methylated derivatives such as choline and betaine must next be considered. Many investigators besides ourselves have shown that these substances increase creatine formation (Abderhalden and Buadze (5), Abderhalden and Möller (6), Baumann and Hines (3), Hongo (1), Riesser (1, 2), Shanks (1) and Sharpe (1)). Stetten (2) showed, from the New York Laboratory, that betaine containing N^{15} was rapidly demethylated to glycine in the rat. Since glycine and the methyl groups liberated both take part in creatine formation, it is readily seen how betaine could act as a methylating agent of glycocyamine. Choline may also do this indirectly through methionine (du Vigneaud (4, 5)). Betaine was not studied by Bloch and Schoenheimer (7), but they stated that choline containing N^{15} was not transferred into creatine containing N^{15} . Here again it is doubtful if this negative result of Bloch and Schoenheimer can be accepted, since the majority of the evidence shows that betaine and choline do increase creatine formation. Kelly and Beard (14) also found that other methylated compounds, such as prostigmin, mecholyl, or caffein, also increased creatine formation and excretion.

Riesser (1, 2) many years ago suggested, without, however, offering any experimental proof up to the present time, that creatine could be formed from choline and betaine with urea, as follows:



This scheme takes on added interest at the present time since Beard and Pizzolato (11) showed that both choline and betaine could react with urea in creatine formation in the rat. Du Vigneaud (3, 5), using heavy carbon, has shown that choline and betaine can transfer one of their methyl groups to the methyl group of creatine and choline. These findings are also of significance with regard to the prevention of fatty infiltration of the liver and hemorrhagic kidneys resulting from a choline deficient diet (Griffith and Wade (1), Griffith (2)).

Stetten (2) fed ethanolamine, choline, glycine and ammonia each containing N^{15} to adult rats. Ethanolamine is a precursor of choline and it arises in the body from dietary glycine even when the diet is poor in methyl groups (3). The major route whereby the animal handles betaine is by a demethylation to glycine and the lipotropic activity of this compound is due to its rôle as a methyl donator.

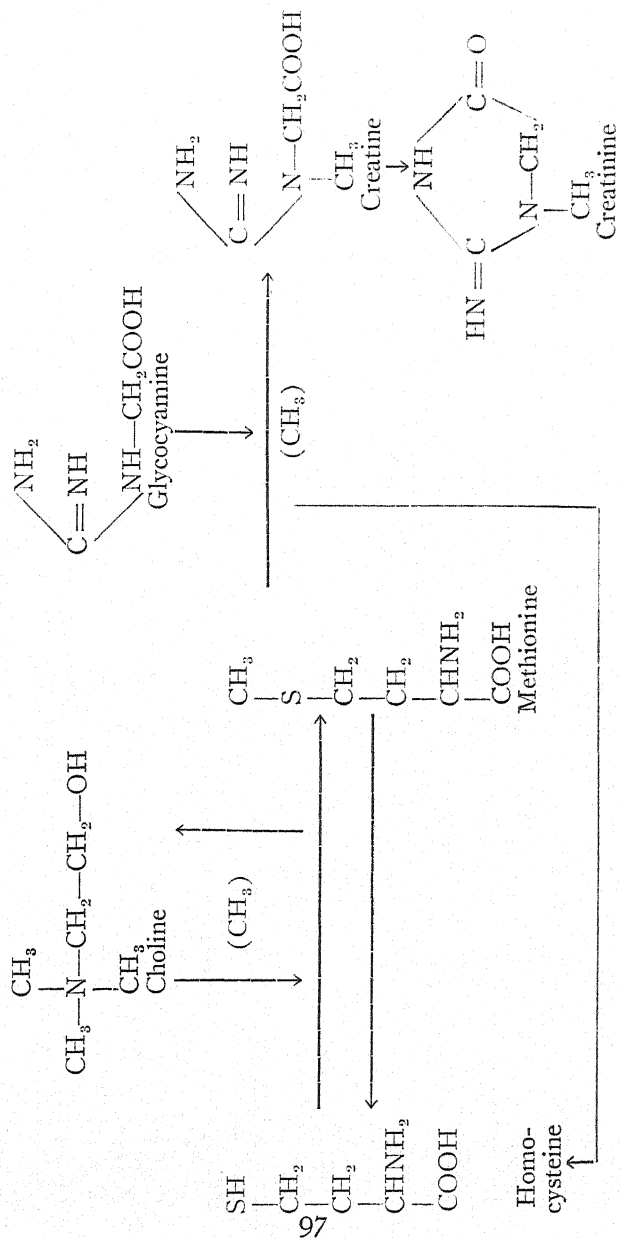
THE RELATION OF THE METHYLATION PROCESS TO STRUCTURE OF THE LIVER AND KIDNEYS

Methylations and demethylations are processes that are known to occur in the body and du Vigneaud (4) has listed the following methyl compounds which occur in animal tissues and excretions:

<i>S-Methyl</i>	<i>Quarternary Nitrogen</i>	<i>Other N-Methyl</i>
Methionine	Choline	Methyl Guanidine
Methyl Mercaptan	Betaine	Dimethyl Guanidine
Dimethyl sulfone	Ergothioneine	Asterubin
Methyl diethyl	Carnitine	Vitiatin
Sulfonium	Neurine	Methyl Amine
Hydroxide	Myokinine	Trimethyl Amine
	γ -Butyrobetaine	Methyl Xanthine
	Stachydrine	Para Xanthine
	Methyl Pyridine	Epiguanine
	Trigonelline	Creatine
	Methyl Quinoline	Creatinine
	Homarine	Sarcosine
	Trimethyl Amine	Epinephrin
	Oxide	Anserine

The investigations of du Vigneaud (4), Griffith (2) and Griffith and Wade (1) in relation to the methyl group have uncovered metabolic interrelationships between the methionine and sulfur metabolism with choline and fat metabolism on the one hand, and between methionine and choline metabolism with creatine metabolism on the other. It is now certain that the methyl group must be supplied in the diet in a utilizable form and that it must be transferred by transmethylation as a unit.

The dietary production of a "fatty liver" in the rat and its prevention by Best and Huntsman (1) in using choline shows the relation of this substance to fat metabolism. Choline is now considered as a vitamin. Betaine (Best and Huntsman (1)), methionine (Tucker and Eckstein (1)), and other compounds (Griffith (2)) show lipotropic activity in older rats and prevent hemorrhagic degeneration in young rats fed on low choline diets. This choline-like action according to Griffith is due to the utilization of the methyl groups of betaine and methionine in the synthesis of choline by the process of transmethylation. Du Vigneaud, *et al.* (2) reported the availability of homocysteine for growth of rats fed upon a methionine-free diet containing betaine or choline and stated that these compounds contained the methyl (CH_3) required for the formation of methionine from homocysteine. Du Vigneaud, *et al.* (1) observed deuterium in tissue choline following the administration to rats of methionine containing deuterium in the methyl group. Hence utilizable methyl groups are essential in the diet. Du Vigneaud (4) draws up the following scheme to illustrate these results:



Griffith (2) has summarized the effects of feeding diets low in choline to rats. After 6 to 8 days the rats may die or a rapid partial recovery occurs. A marked increase in liver fat occurs in 2 days and this indicates that the rats have no reserves of choline. The increase in liver lipids reaches its maximum after 4 to 6 days and during this time there is no interference with growth or appetite. The liver fat at this time may be as much as 25 to 30 per cent of the weight of the liver. Renal degeneration occurs between the 6th and 8th days with a marked elevation of blood non-protein nitrogen. There is also a regression of the thymus and ocular hemorrhage. The renal changes usually occur within 1 or 2 days and are characterized grossly by an increase in size and weight and by hemorrhagic discoloration. Proteinuria, but not hematuria, occurs. There is also a diminished rate of excretion of phenol red. This is related to the N.P.N. of the blood and the appearance of phenol red in the kidneys. The inulin excretion of normal rats is about 75 per cent during the first three hours after injection. In the choline deficient rats this rate is about 20 per cent.

A histological examination of the kidneys of Griffith's rats was made by Dr. K. Christensen of St. Louis University. The important renal changes were described as follows:

"Vascular congestion and tubular degeneration are observed microscopically in kidneys of rats receiving the choline deficient diets. In the most severe cases, necrosis occurs in the peripheral part of the cortex including the vascular system, renal tubules, and interstitial tissue. The enlarged and deep red appearance of the kidneys is caused primarily by the congestion of the peripheral cortical capillaries and the capsular blood vessels. Glomerular and other renal blood vessels appear normal, but in severely affected animals glomeruli in the peripheral cortex are also congested. Evidence of hemorrhage, if present, is found only in the capsule and at the edge of the cortex. Blood in the

outermost part of the cortex may come from ruptured blood vessels of the capsule. Numerous fat droplets can be demonstrated histologically in the blood of the congested cortical vessels. Except in the kidneys having extensive cortical necrosis, damage to the vascular system is slight. Affected parts of renal tubules in the cortex especially the convoluted portions, show necrosis, albuminous degeneration, or 'hyalin droplet' degeneration. In some of the tubules with albuminous degeneration, edema and desquamation are found. The necrosis of convoluted tubules occurs in the same areas of the cortex as vascular congestion. Fat is abundant in the bases of the necrotic cells. When the degeneration is less intense, the convoluted tubules are enlarged and the cytoplasm of the cells is broken up into fine granules or collected into hyaline globules, either of which may fill the lumina of the tubules. An albuminous precipitate is present in some of the Bowman's capsules. Tubules in the deep part of the cortex and the outer part of the medulla are always filled with casts. More superficially in the cortex, scattered tubules have casts and in severely affected animals casts are present in the tubules and papillary ducts of the renal pyramids.

Closely following the acute reaction, the interstitial tissue of the peripheral cortex proliferates and richly cellular connective tissue is formed which closely surrounds the degenerated renal tissue as well as the parts beginning to regenerate. Leucocytic infiltration of the connective tissue is never abundant. During the stage of fibrosis, the kidneys grossly show the 'frosted' appearance and calcification of the degenerated tubules may be observed. In the recovery phase blood leaves the congested vessels; necrotic and calcified tubules are removed by lysis; tubular regeneration is attempted in partially necrotic tubules; cytoplasm is restored in non-necrotic tubules; and macrophages take up the free red blood corpuscles in the most peripheral cortex and in the capsule. Casts disappear from the tubules in both the cortex and medulla. Several months after the acute phase the regenerated kidneys resemble normal kidneys in structure. The repair of the tubules which have undergone albuminous degeneration especially helps to reestablish the peripheral cortex of the regenerated kidney. Some kidneys, however, continue to show mild chronic degeneration."

These choline deficiency symptoms can be prevented or abolished by feeding choline. The renal lesions are prevented by 1 to 2 mg. per day while the fatty liver is prevented by 4 to 6 mg. per day. The methyl group of methionine is very effective, but only one of the methyls of betaine is effective, since this compound is only about one-third as effective as choline.

In summary it may be stated that while the mechanism of the biological process of methylation of glycocyamine to creatine is not clear, the results listed above definitely establish the fact that no one compound, such as methionine, can be considered as specific in this connection. The process of methylation may be specific, but there are several compounds that can furnish the methyl group to take part in this biological reaction or stimulate it in the body. The production of fatty livers on low choline diets with kidney degeneration also suggests that methylation is a most important biological process and further indicates that a source of these methyl groups must be present in the diet in order to prevent these pathological changes.

SITE OF CREATINE, CREATININE AND GLYCOCYAMINE FORMATION IN THE BODY

The formation of these compounds in the body is evidently due to enzymic processes. This at once implicates the liver, kidneys and muscles as the most likely sites for the production of these substances in the body. The evidence for this theory listed in Hunter's monograph (1), however, is not impressive for any of these tissues. It is practically impossible to determine the site of creatine formation in the intact body due to the large number of variables encountered. But many *in vitro* studies have been conducted which will throw some light on this interesting question (Table 15).

TABLE 15

POSSIBLE SITES OF GLYCOCYAMINE AND CREATINE
FORMATION FROM THEIR PRECURSORS

<i>Tissue</i>	<i>Type of Experiment</i>	<i>Observer</i>
Liver	Liver perfusion	Inouye (1)
Liver	Liver autolysis	Myers and Fine (3)
Liver, muscle	Liver and muscle autolysis	Gottlieb and Stangassinger (1)
Liver	Liver perfusion with blood	Gottlieb and Stangassinger (1)
Muscles	Muscle autolysis	Hoagland and McBryde (1)
Muscles	Muscle perfusion	Dorner (1)
Muscles	Muscle perfusion	Seeman (1)
Muscles	Muscle perfusion	Palladin and Wallenburger (2)
Muscles	Muscle perfusion	Baumann and Hines (2, 3)
Heart	Glycocyclamine + glycine	Bach (1)
Liver, kidney, heart, diaphragm and small intestine (rat)	Incubated in Ringer's solution	Borsook and Jeffreys (6)
Liver (rat)	Incubated with protein hydrolysate	Borsook and Jeffreys (6)
Liver slices from cat, rabbit and rat	Glycocyclamine in Ringer's solution	Borsook and Dubnoff (2)
Liver slices (rat)	Glycocyclamine + methionine in Ringer's solution	Borsook and Dubnoff (2, 3, 4)
Kidney slices (rat), beef, cat, dog, guinea pig and sheep	Arginine + glycine	Borsook and Dubnoff (4)
Liver	Incubation and perfusion with arginine, glycine, glycocyclamine, etc.	Shibuya (1)
Liver	Perfusion with alanine, arginine, thyroxine, etc.	Shibuya (1)
Liver	Cyanamide, glycine and ammonia	Shibuya (2)
Kidney, heart, liver and muscle (pigeon)	Arginine + glycine	Borsook and Dubnoff (4)
Heart	Perfusion with Ringer-Locke solution with arginine	Fisher and Wilhelmi (2)
Heart	Perfusion with Ringer-Locke solution, glycocyclamine and glycollic acid	Davenport, <i>et al.</i> (3)
Muscle	Incubation of muscle tissue with urea and glycine, urea and sarcosine, cyanamide with glycine and cyanamide with sarcosine	Beard and Espenan (21)
Kidney and liver	Incubated in phosphate or bicarbonate buffer	Baker and Miller (2)

The above results reveal that tissues of different animals vary in their ability to form glycocyamine and to methylate it to creatine. They also indicate that the mechanism for creatine synthesis from urea and glycine resides in the muscles, while that for arginine and glycine resides in the kidney, and that methylation takes place in the liver. That the synthesis of creatine is, however, not limited to the muscles, liver and kidneys, is shown conclusively by the results obtained by Baker and Miller (2) which show that creatine formation occurs in most tissues studied and by Borsook and Dubnoff (4) who showed that glycocyamine is widely distributed in animal tissues. Creatine can also be formed in the dog in the complete absence of the liver (Beard (9)).

It may, therefore, be concluded on the basis of the above evidence, that the muscles, liver and kidneys are the tissues chiefly concerned with the formation of creatine and creatinine from most of the amino acids of the diet. It is also certain that creatine can arise from creatinine *in situ* in the body and also in *in vitro* studies (Cf. Chapters VIII and IX).

CHAPTER VIII

CREATINE-CREATININE RETENTION AND EXCRETION. THE ORIGIN OF CREATININE. CREATININE-PHOS- PHORIC ACID. CREATINE TOLERANCE

CREATINE EXCRETION occurs in the urine of children up to the time of puberty. This is evidently due to an increased production of creatine from its precursors during this time. It is well known that the protein intake during growth is much greater than it is after adulthood has been reached. Also, injection of the hormones of the thyroid and anterior pituitary, especially the anterior pituitary growth hormone, induces creatinuria, and these hormones are very active during the growth period.

Creatinuria is also of common occurrence in women, especially during pregnancy (Krause and Cramer (1, 2); van Hoogenhuyze and ten Doeschate (1); Heynemann (1); Wakulenko (1); Murlin (1); Murlin and Bailey (2)). Otherwise the creatinuria is due to protein ingestion (Daniels and Hejinian (1); Denis (2); Denis and Kramer (3); Folin and Denis (7); Gamble and Goldschmidt (1) and Wang and Kaucher (1)).

Only a few studies have been made upon the creatine excretion in normal children. Wilkins, *et al.* (1, 2, 3, 4) have summarized the most significant studies in this connection (Table 16).

TABLE 16
CREATINE EXCRETION OF DIFFERENT AGE GROUPS
REPORTED BY VARIOUS WORKERS
(After Wilkins, *et al.* (1, 2, 3, 4))

Age	No. of Cases	CREATINE		Observer	Diet	Study, days
		Average, mg./kg./day	Range, mg./kg./day			
Prematures	6	Almost none		Marples & Levine (1)	Milk	Part of 1
1-8 weeks	5	Almost none		Paffrath & Ohm (1)	Milk	1
15-20 weeks	41	4.6	Not given	Catherwood & Stearns (1)	Milk	1
2-7 months	36	10.5	Not given	Catherwood & Stearns (1)	Milk	1
1½-7 months	11	10.3	4.4-18.9	Daniels & Hejninian (1)	Milk	3
5-12 months	5	11.8	6.0-15.3	Daniels & Hejninian (1)	Milk	1-5
2½-3½ years	6	17.3	6.8-22.2	Roughchitch (1)	Milk	2
4-4½ years	4	8.8	7.5-9.6	Harding & Gaebler (1)	Low creatine	2
9-9½ years	4	5.1	4.2-5.7	Harding & Gaebler (1)	High protein	3
5-11 years	4	4.2	1.0-6.0	Harding & Gaebler (1)	Low creatine	3
(boys)	13	4.9	1.4-11.2	Shelton & Tager (1)	Low creatine	2
6-13 years	18	7.1	2.4-15.3	Shelton & Tager (1)	Low creatine	2
(girls)						

It has generally been believed that the normal adult man fed upon a creatine and creatinine free diet does not excrete any creatine. In recent years, however, evidence has accumulated to show that this view is no longer tenable (Beard (32)). Djen and Platt (1) showed that 108 out of 148 normal Chinese subjects showed creatinuria and, in many cases, a remarkably low creatinine output. Hobson (1) found a mean value of 637 mg. (92 to 1,200 mg.) creatine in 97 normal male urine specimens. These were from physical training students fed on diets rich in carbohydrates. When the latter were omitted from the diet the creatinuria disappeared. These results confirm those of Haldi and Bachmann (1) who showed that the ingestion of glucose or fructose would result in creatinuria in man. The increases in the color of the Jaffé reaction in the studies of Hobson and Haldi and Bachmann were not due to glycosuria. V. Moraczewski and Grzycki (1) also observed that sugar feeding would increase creatine excretion. Taylor and Chew (1) reported the excretion of 0 to 196 mg. creatine per day in 15 adult males. E. Wang (1) observed a maximum excretion of 100 mg. of creatine in the urine of male patients and 200 mg. in the urine of women patients. Dill and Horvath (1) also observed an excretion of creatine in the urines of their 4 normal subjects. It is, therefore, concluded that creatine may appear in small quantities in the urine of adult males.

The output of creatinine was supposed to be constant for a given individual if the diet is free of creatine and creatinine. Folin even stated that the accuracy of the 24 hour collection of urine could be estimated by its creatinine content. These views of Folin are, however, no longer tenable. One of the most interesting developments from our studies and those of others is that this constancy is only relative and that *wide variations* in creatine and creatinine excretion may

and usually do occur in a given individual or in different individuals. Some of the other investigators who have noticed these variations are Cameron (1), E. Wang (1), Espersen and Thomsen (1, 2), Thomsen (3), Dill and Horvath (1) and Wilkins, *et al.* (1). Espersen and Thomsen concluded that the day to day variations in creatine and creatinine excretion are so great in some cases of myopathies that no conclusion could be drawn from the action of glycine in these cases. This statement will, however, hardly be accepted by students of creatine and creatinine metabolism.

TABLE 17
FATE OF INGESTED CREATINE
(After Folin (8))

<i>Creatine Given, gm.</i>	<i>Total N, gm.</i>	<i>Urea N, gm.</i>	<i>Creat- inine, gm.</i>	<i>Extra Creat- inine, gm.</i>	<div>CREATINE</div> <div><i>Excreted, Retained,</i> gm. gm.</div>	
	5.5	4.2	1.3	0.0		
0.97	5.4	3.9	1.3	0.0	0.0	0.97
	4.0	2.8	1.3	0.0		
4.40	3.9	2.4	1.8	0.0	0.84	3.56
(1 dose)	3.7	1.8	1.7	0.0		
	3.8	2.3	1.8	0.0		
4.40*	19.9	16.4	1.5	0.0	2.34	2.06
(3 doses)	21.7	17.7	1.5	0.0		
	20.2	17.0	1.4	0.0		

* High protein diet.

When creatine or creatinine is ingested, their quantitative recovery in the urine is never obtained. It is possible that different amounts of these substances may be destroyed by bacterial action in the intestines. From 20 to 80 per cent cannot be accounted for after their ingestion by mouth (Table 17). Since creatine is stored only in small amounts in the tissues, and since creatinine is not stored at all, it is evident that these substances are undergoing some transformations into other substances. As mentioned above, Kapellar-Adler

and Toda (1) showed that methylamine was excreted into the urine after the administration of creatine. Guggenheim and Löffler (1) found from 2 to 10 mg. of trimethylamine per liter of normal human urine and Doree and Golla (1) found trimethylamine in normal human blood and cerebrospinal fluid. Tarr (1) showed by both biological and chemical methods the presence of 110–250 mg. of trimethylamine oxide dihydrate in the normal urine of individuals who had ingested a fish-free diet. After ingestion of trimethylamine its oxide was excreted into the urine.

Methyl guanidine and guanidine itself have been isolated from the urines of some myopathy patients. It is possible, therefore, that Schoenheimer's statement that creatine and creatinine undergo no transformations in the tissues, except the conversion of creatine into creatinine, is untenable. The tracer technique is not adequate to show all of the possible changes that these substances may undergo in the body, unless all possible end products are studied.

TABLE 18
EFFECT OF PARENTERAL INJECTION OF CREATINE UPON
CREATINE-CREATININE EXCRETION
(After Beard and Jacob (17))

<i>Creatine Injected, mg.</i>	<i>Creatine "as Creat- inine" Excreted, mg.</i>	<i>Creatine "as Creat- inine" Retained, mg.</i>	<i>Creatine "as Creat- inine" Retention, Per Cent</i>	<i>Creatinine Retention in Relation to Creatine Injected, Per Cent</i>	<i>Creatinine Retention, mg.</i>	<i>Duration of Experi- ment, Days</i>
25	20.1	4.9	24.5	300.0	74.0	12
50	20.6	29.4	58.8	121.0	60.5	10
75	18.2	56.8	75.7	92.0	68.8	12
100	45.0	55.0	55.0	69.5	69.5	12
150	75.0	75.0	50.0	54.6	82.0	10
200	76.0	124.0	62.0	31.5	63.3	12

Beard and Jacob (17) reported that the retention of creatine varied from 20 to 75 per cent when doses from 25 to 200 mg. of creatine were injected. The *creatinine retention*, in

relation to the dose of creatine injected, varied from 300 per cent for the 25 mg. dose to 32 per cent for the 200 mg. dose (Table 18). In man even more striking results were obtained. Ingestion of 3 to 5 gm. of creatine by 6 normal subjects caused a creatine retention varying from 0 to 37 per cent of the dose ingested. The creatinine retention varied from 0 to 3 gm. In all of these studies creatine was not transformed into creatinine (Table 19).

TABLE 19
EFFECT OF CREATINE INGESTION UPON CREATINE-
CREATININE METABOLISM
(After Beard and Jacob (17))

Subject	Creatine Ingested, gm.	Creatine Excreted, gm.	Creatine Retained, gm.	Creatine Retention, Per Cent	Creatinine Retention, gm.	Duration of Experiment, Days
P. Pt.	5.0	5.0	None	None	3.0	12
	5.0	4.0	1.0	20	None	14
H. H. B.	3.0	1.9	1.1	36.6	None	8
P. Pz.	3.0	2.4	0.6	20	0.6	8
E. J.	5.0	5.4	None	None	0.8	16
S. G.	3.0	2.9	0.1	33.3	1.7	12
C. J. K.	5.0	4.8	0.2	4	0.4	10

When creatinine in doses from 10 to 200 mg. was injected into young rats the creatinine retention varied from 0 to 124 mg. accompanied by an increased creatine excretion of 23 to 60 mg. (Table 20). The ingestion of 0.5 to 5 gm. of creatinine by the same 6 individuals discussed above caused a retention of creatinine varying from 1 to 3 gm. (Table 21). Creatinine may, therefore, be excreted or retained depending upon the experimental subject. The increased *creatinine excretion* under these conditions was striking, varying from 5 to 20 gm. These unusual results have been obtained in several other studies (Beard, *et al.* 18, 19, 22, 23, 24). The color of the Jaffé reaction of all the urines tested was destroyed by the creatinine enzyme of Miller and Dubos.

TABLE 20

EFFECT OF PARENTERAL INJECTION OF CREATININE
UPON CREATINE-CREATININE EXCRETION
(After Beard and Jacob (17))

<i>Creatinine Injected, mg.</i>	<i>Extra Creatinine Excreted, mg.</i>	<i>Extra Creatinine Retained, mg.</i>	<i>Creatine "as Creatinine" Excreted, mg.</i>	<i>Conversion of Retained Creatinine into Creatine, Per Cent</i>	<i>Duration of Ex- periment, Days</i>
10.0	None	10.0	48.1	481.0	16
20.0	None	20.0	29.6	148.0	20
30.0	9.5	20.5	29.3	143.0	16
40.0	25.0	15.0	23.0	153.0	20
50.0	28.0	22.0	40.1	182.0	16
60.0	29.0	31.0	28.8	93.0	20
75.0	39.0	36.0	30.0	83.3	8
100.0	55.0	45.0	40.0	88.8	8
150.0	35.0	115.0	41.0	35.6	8
200.0	76.0	124.0	60.0	48.3	8

TABLE 21

EFFECT OF CREATININE INGESTION UPON CREATINE-
CREATININE METABOLISM
(After Beard and Jacob (17))

<i>Subject</i>	<i>Creat- inine Ingested, gm.</i>	<i>Extra Creat- inine Excreted, gm.</i>	<i>Extra Creat- inine Retained, gm.</i>	<i>Creatine "as Creatinine" Excreted, gm.</i>	<i>Conversion of Retained Creatinine into Creatine, Per Cent</i>	<i>Duration of Ex- periment, Days</i>
P. Pt.	0.5	0.1	0.4	None	None	2
	1.0	9.5	None	20.2	None	18
	3.0	8.5	None	8.3	None	14
	5.0	7.9	None	5.3	None	8
H. H. B.	1.0	3.7	None	10.2	None	10
	3.0	7.9	None	8.4	None	12
	5.0	4.7	0.3	9.4	3,133	24
	1.0	3.7	None	6.4	None	12
P. Pz.	0.5	3.4	None	None	None	2
	1.5	None	1.5	9.6	640	12
	3.0	5.5	None	6.0	None	10
	5.0	5.8	None	6.1	None	20
	1.0	0.4	0.6	7.4	1,230	16

TABLE 21 (Continued)
EFFECT OF CREATININE INGESTION UPON CREATINE-
CREATININE METABOLISM
(After Beard and Jacob (17))

Subject	Creat- inine Ingested, gm.	Extra Creat- inine Excreted, gm.	Extra Creat- inine Retained, gm.	Creatine "as Creatinine" Excreted, gm.	Conversion of Retained Creatinine into Creatine, Per Cent	Duration of Ex- periment, Days
E. J.	0.5	0.6	None	None	None	2
	1.0	9.8	None	8.9	None	10
	2.0	None	2.0	6.8	340	22
	5.0	4.6	0.4	5.0	1,250	20
S. G.	0.5	0.2	0.3	2.2	733	8
	1.5	6.8	None	7.1	None	10
	3.0	None	3.0	8.2	273	16
	3.0	2.1	2.9	2.8	100	8
	1.5	5.0	None	5.4	None	14
C. J. K.	0.5	0.1	0.4	None	None	2
	1.0	9.7	None	21.0	None	14
	3.0	2.0	1.0	10.6	1,060	18
	1.0	1.6	None	7.7	None	26

The results of these studies also reveal a new feature of creatine and creatinine metabolism, namely, that the injection or ingestion of creatinine in the rat or man *greatly stimulates* the formation and excretion of creatine and creatinine in the body. So large were the increases in creatine excretion after the injection of small doses of the purines and their methylated derivatives that Beard, *et al.* (15, 16) concluded that a stimulation of creatine formation and excretion occurred under these conditions (Tables 22 and 23).

Bachmann, *et al.* (2) in a study of the effect of caffeine on the respiratory exchange in man found creatine in the urine in 30 out of 35 experiments. The amounts of caffeine ingested varied from 1.8 to 6 mg. per kilo body weight. In 4 experiments the creatine excretion varied from 0.7 to 1 mg. per hour and in the remainder from 1.1 to 3.3 mg. per hour. No

TABLE 22
EFFECT OF CAFFEIN INJECTION ON CREATINE-CREATININE METABOLISM
(After Beard and Pizzolato (15))

Number of Animals	Substance Injected, mg.	Experi- ment, Days	MUSCLE CREATINE			TOTAL CREATININE			URINE PREFORMED CREATININE			CREATININE- decrease		
			Nor- mal, mg. per gram	Experi- mental, mg. per gram	Increase, Per Cent	Average Control Days, mg. per 2 Days	Average Experi- mental Days, mg. per 2 Days	Increase, Per Cent	Average Control Days, mg. per 2 Days	Average Experi- mental Days, mg. per 2 Days	Increase, Per Cent	Average Control Days, mg. per 2 Days	Average Experi- mental Days, mg. per 2 Days	In- crease, Per Cent
29	Controls	4	4.2	10.1	140	14.1	26.6	88	13.2	17.6	33	1.0	10.4	940
		6		9.5	126		19.5	38		8.3	None		13.0	1,200
		8		8.0	90		28.7	103		7.8	None		24.2	2,320
12	Caffein 4.0	10		6.3	50		14.3	None		9.4	None		5.7	470
		12		4.3	None		17.4	23		14.1	6		3.8	280
		14		4.2	None		14.2	None		13.0	None		2.0	100
29	Controls	2	4.2	13.0			37.4	188	12.4	17.0	37	0.7	23.7	3,286
		4		6.2	76		20.5	57		11.4	None		10.6	1,414
		6		7.7	83		29.1	123		21.2	71		9.2	1,214
12	Caffein 4.0	10		6.0	42		37.3	186		24.8	100		14.5	1,971
		12		3.7	None		21.2	63		12.6	None		10.0	1,328
		14		3.9	None		15.3	17		8.7	None		7.7	1,000
		16												

TABLE 23

EFFECT OF THEOBROMINE AND XANTHINE INJECTION ON
CREATINE-CREATININE METABOLISM
(After Beard and Pizzolato (15))

Number of Animals	Substance Injected, mg.	Experi- ment, Days	MUSCLE CREATINE			TOTAL CREATININE				URINE PREFORMED CREATININE				CREATINE			
			Nor- mal, mg. per gram	Experi- mental, mg. per gram	Increase, Per Cent	Average Control Days, mg. per 2 Days	Average Experimental Days, mg. per 2 Days	Increase, Per Cent	Average Experimental Days, mg. per 2 Days	Average Control Days, mg. per 2 Days	Increase, Per Cent	Average Experimental Days, mg. per 2 Days	Average Control Days, mg. per 2 Days	In- crease, Per Cent	Average Experimental Days, mg. per 2 Days	Average Control Days, mg. per 2 Days	In- crease, Per Cent
29	Controls	2	4.2														
6	Theobro- mine 4.0	4		6.0	42	13.9*				13.4*			0.6*		10.4		1,633
		6		8.2	95		21.1	51			12.1				37.5		6,150
		8		5.9	40		45.5	226			13.2				2.4		300
		10					18.2	30			16.1				1.5		150
		12					12.6	None			11.3						
29	Controls	2	4.2														
6	Xanthine 4.0	4		5.9	40	13.9*				13.4*			0.6*		4.2		600
		6		8.2	95		17.8	28			14.2				25.6		4,166
		8		6.7	59		34.1	145			12.0				2.6		333
		10					13.2	None			10.8				1.7		183
		12					12.6	None									

* These are the average excretions in mg. per 2 days of the different creatine bodies for the 29 control animals.

definite relationship was observed between the amount of caffeine ingested and the rate of creatine excretion. The analyses were made one hour and forty-five minutes after ingesting the caffeine. In these studies there was also no relation found between the total oxygen consumption and the amount of creatine excreted.

Of much interest in this connection are the findings of Hess and Sullivan (2) who showed that the ingestion of alanine, glycine or glutamic acid by a cystinuric patient caused a big stimulation of the excretion of the sulfur containing amino acid cystine. In closing his review on "Intermediate Metabolites and Respiratory Catalysts," Elliott (1) made this statement: "More than ever biochemists need to have the biological outlook, the recognition of the complex interlocking of dynamic systems in biological materials." This means that biochemists should not forget the many factors that can alter the rate of chemical reactions in the body. This statement is certainly applicable to studies in creatine and creatinine metabolism.

A possible clue to the large retentions of both creatine and creatinine in man observed in our studies discussed above is offered in the recent studies of the author (Beard (37)). In these *in vitro* studies it was shown that large amounts of creatine and creatinine, in contact with various tissues in a phosphate buffer solution, disappeared (Table 24). It is possible that these substances were oxidized to other products. We may be measuring the oxidation rather than the retention of creatine and creatinine under these conditions. The "disappearance" of creatine which was mentioned by many workers in the past might likewise have been creatine that was oxidized. Even though Schoenheimer and others have shown that the methyl group of creatine is stable in the body the above evidence is very convincing that both creatine and creatinine can be oxidized into other substances in the tissues.

Also the creatinine enzyme of Miller and Dubos oxidizes both creatinine and sarcosine and it is, therefore, possible that sarcosine is an oxidation product of creatinine in the tissues. In this reaction the methyl group of creatinine would not be destroyed in the tissues, but it would be shifted by the "trans-methylation" reaction to sarcosine, or some other substance, *e.g.*, guanidine.

Schoor and Boer (1) stated that the spontaneously existing creatinuria in normal children is increased by giving them 1 gm. doses of creatinine. In uremia, Linegar, *et al.* (4) found an increase in heart and skeletal muscle creatine, phosphorus and potassium, and stated that by retention of creatinine the equilibrium between creatine and creatinine would be shifted toward the former.

E. Wang (1) reviewed the entire subject of creatinuria. His experimental work upon blood, urine, and muscles of animals and patients showed that creatinuria was produced under a wide variety of conditions. He concluded that creatinuria was an entirely unspecific phenomenon. His rabbit studies show that, in experimental thyreotoxicosis produced by injections of thyroxine, the creatine and especially the creatine phosphate content, is much lowered. Intravenous injection of creatine does not change the creatine and creatine phosphate content of the muscles and when it is injected with thyroxine the effect is no different than in rabbits injected with thyroxine alone. The muscle creatine and creatine phosphate content of the thyroidectomized rabbits is somewhat higher than in normal animals. It is, therefore, concluded that the creatinuria was due to a loss of muscle creatine.

Thomsen (3) also reviewed the subject of creatinuria, including his own contributions to the subject. In order to decide whether, in a given case, creatinuria is present, the quotient,

$$\frac{\text{total creatine}}{\text{preformed creatinine}} \times 100$$

is calculated, and is called the "creatinine index." Only when this index exceeds 105, can creatinuria be diagnosed by his technique.

Thomsen concluded that the normal creatinine content of the blood is about 1 mg. per 100 cc. of plasma. He doubts if the plasma contains creatine when the subject is fed upon a creatine-free diet. Creatine was fed by mouth to guinea pigs and man. The results of these studies indicated that creatine is a threshold substance which in man is about 1.25 mg. of creatine per 100 cc. of plasma. Thomsen upholds Shaffers' theory to account for endogenous creatinuria. This creatine arises from that liberated from muscle tissue and the creatinuria is accompanied by a degeneration of muscle tissue (Myers and Fine (1)).

This explanation of endogenous creatinuria does not, however, in the author's opinion, tell the whole story. There are many cases where endogenous creatinuria occurs before any tissue degeneration is present. The creatinuria here is due to the fact that glycogen breakdown is at a minimum. This causes a lack of phosphate donators from hexosephosphates and consequently increased creatine formation is greater than can be phosphorylated in the muscles. The extra creatine is then excreted into the urine. It is only after muscle degeneration occurs that creatine is excreted as such in the urine. Creatinuria, or lack of it, therefore, would depend primarily upon the supply of phosphate donators in the tissues. This again, however, does not tell the complete story since phosphate transfer to creatine is a complex reaction involving adenylic acid, its phosphorylation products and numerous other coenzymes and metabolites.

Thomsen believes that the creatinuria of children is largely

due to the creatine content of the milk ingested. Children fed upon milk-free diets still excrete creatine (Harding and Gaebler (1), Shelton and Tager (1)). Human milk does not contain very much creatine. Hence Thomsen found that creatinuria seldom occurred in breast fed infants. Breast milk contains 0.5 mg. of creatine, and cows' milk, 5 mg. of creatine per 100 cc. Creatinuria was stated not to be a normal finding in young animals. The excretion of 10 to 20 mg. of creatine per 3 day period has occurred in hundreds of control animals in the author's studies.

The excretion of creatine in the urine of both young and old subjects has been studied by numerous investigators. This creatine may be produced from disintegrating protein; by a disturbance in carbohydrate utilization; in starvation; by a disturbance in almost any of the endocrine glands; in various pathological conditions, such as fevers, diabetes mellitus, exophthalmic goiter; and in the myopathies. Under normal conditions, however, it is very easy to show a *new production and excretion* of the excess creatine from its various precursors in the diet. Mitchell and Hamilton (1) have discussed this aspect of the subject and subscribe to the view that creatinuria in many cases is due to an accelerated endogenous production of creatine in the tissues. In starvation the excretion of nitrogen and creatine roughly parallel each other (Benedict (1), Howe (1)).

There are at least two general theories which have been advanced to explain the appearance of creatine in the urine. Myers and Fine (1) believe that urinary creatine arises from disintegrating muscle tissue, with a liberation of its creatine. Brentano (1-7) on the other hand believes that it arises from a breakdown of muscle glycogen.

The author does not subscribe entirely to the theory of Myers and Fine. It is of course conceivable that disintegrating muscle tissue would liberate some creatine which would

then, in the absence of phosphate donors, be excreted into the urine. But one has only to take the analogous case of the creatine excretion in the muscular dystrophy patient to see that their theory is untenable. Assume that a normal adult male has 120 gm. of creatine in his muscles. Later this same individual develops muscular dystrophy and excretes about 0.6 gm. of creatine daily. It is clear that, should the muscle creatine itself be the mother substance of this urinary creatine, the patient would excrete (with the exception of the newly formed creatine from amino acids) all of his body creatine into the urine in about 200 days. Since the creatine excretion of this type of patient is extremely variable and he may live as long as 20 or 30 years with this disease, some other explanation must be found for the creatinuria. It is now known that the defect in this type of patient is not one of the synthesis of creatine, since creatine formation is normal, but is one of the *utilization* of creatine. Hence the newly formed creatine is not utilized, especially in the area of the diseased muscles, but is then excreted as such in the urine. Since there is also a defect in carbohydrate metabolism in the patient, it is possible that some of the creatinuria might be due to a lack of phosphate donors in the muscles to hold the creatine as creatine phosphate.

Brentano (1, 2, 3, 4, 5, 6, 7) in a series of publications has suggested the theory that most types of creatinuria are due to a breakdown of muscle glycogen. He found a decrease in muscle glycogen in several conditions that produce creatinuria. Jahn (1, 2) has reached similar conclusions. The writer does not subscribe to this hypothesis. In the first place creatinuria can occur in several conditions without a diminution of muscle glycogen. In the second place the injection of epinephrin gives a *glycosuria* and not creatinuria, since the color of the Jaffé reaction in these cases was not destroyed by the creatinine enzyme of Miller and Dubos. The writer's

theory is that any condition that causes a loss of muscle phosphate will result in creatinuria, since there will be a lack of phosphate donors for creatine to form creatine phosphate under these conditions. This theory, however, does not apply to an overproduction of creatine, with subsequent creatinuria, from its precursors in the diet (Cf. Chapter XIV).

Mourot (1) made an extensive study of the nature of the excretory products resulting from the catabolism of the amino acids in both young and old rats. Most of these, and especially arginine, histidine, aspartic acid and glycine, caused an increase in creatine excretion, while the excretion of creatinine was not changed. She suggested that glycine may either (a) methylate an intermediate product formed from arginine or histidine which contained the guanidine group, or (b) be directed from its normal path and combined with a precursor of urea (cyanic acid, Werner (1)) or with a tautomeric form of urea which Werner has shown to be active. Kelly and Beard (14) observed an increase in creatine excretion when methyl amine, methyl urea, mecholyl, caffeine, prostigmin, etc., and also when such methylating agents as glycine, glycollic acid, methyl alcohol and para-formaldehyde, were injected.

Folin (1, 2, 3) stated that the creatinine output was not influenced by protein feeding. This is an erroneous view that has been widely accepted (Cf. page 39). Even if this statement were true, it does not follow that the creatinine output will always be constant when *increased amounts* of the proteins and amino acids are fed or injected.

The large amount of evidence for the amino acids as precursors of creatinine was reviewed by Boggess and Beard (10). An increase up to 138 per cent in creatinine excretion was observed in 60 per cent of their animals when extra large doses of glycine or arginine were injected. Extra creatine excretion did not occur in these studies. The size of the dose

of amino acids injected is important, small ones increasing creatine and larger ones creatinine, excretion. These results confirm those of Beard and Barnes (3) in 1927 in which most of the amino acids and proteins ingested brought about an increased elimination of creatinine in the rat and man. Goudsmit (1) and Zacherl (1) observed lower values for renal venous blood as compared to renal arterial blood and this indicates that the blood creatinine is the immediate precursor of urine creatinine.

Beard and Pizzolato (11) observed that the injection of urea and glycine into rats gave over twice as much creatine formation in their muscles as compared to the injection of either alone (Table 11). Since this increased creatine storage lasts for only two days, it is to be expected that some of the overflow creatine would find its way into the urine. The same might also be true of creatinine. Beard, Espenan and Pizzolato (12) fed urea and glycine, alone and together, to rats and to two adults. Creatine excretion was directly proportional to the amount of these substances fed separately or together in the diet. Subject HHB excreted 35.5 gm. of *extra* creatine and creatinine, as compared to the theoretical amount of 36.5 gm., when 5 gm. each of urea and glycine were ingested. In a second study this same individual excreted 24.5 gm. of extra creatine and creatinine. Subject PP, under similar experimental conditions, excreted about one-fourth as much creatine and creatinine as did subject HHB.

Beard (13) repeated this study using sarcosine, or methyl glycine, in place of glycine. In the rat the injection of sarcosine or urea alone or together caused an increased creatine excretion of 50 to 71 mg., while the injection of sarcosine alone gave 114 mg. of extra creatinine excretion. In the case of subject HHB, the ingestion of sarcosine or urea alone gave no increase in creatinine excretion, but the *extra* creatine excretion was 6 to 13 gm., respectively. But when sarcosine

and urea were ingested together, the *extra* creatine excretion was 12 gm. and creatinine excretion 29 gm., or a total excretion of both creatine and creatinine of 41 gm., which was 6 gm. more than required by theory and 6 gm. more than was excreted under identical conditions in the same subject with urea and glycine. Here again one sees the effect of the methyl group of sarcosine on creatine-creatinine formation and excretion. Evidence was obtained in these studies with the rats that the increase of creatinine excretion occurs in some cases before that of creatine. It is, therefore, very likely that creatinine was an intermediate compound in creatine formation in these cases. In any case, ammonia, urea, uric acid and creatinine should no longer be considered entirely as waste substances of metabolism. Their effect on creatine and creatinine formation serves a very useful purpose in the body.

The following substances have been shown to increase creatine or creatinine excretion after their ingestion:

Glycine: Udeles and Shretter (1, 2); Paschis and Schwoner (1, 2); Degan (1); Barr (1); Braestrup (1); Mader, *et al.* (1); Brand, *et al.* (1-6); Adams, *et al.* (2, 3); Allinson, *et al.* (1); Bech (1); Boothby (4, 5); Cuthbertson and Maclachlan (1); Hellich and Tessenow (1); Hines and Knowlton (5); Kisner, *et al.* (1); Kostakow and Slauck (1, 2); Lineweh and Lineweh (1); Milhorat, *et al.* (2-6); Perrier (1); Reich (1); Reinhold, *et al.* (1); Schoor and Boer (1); Shorr, *et al.* (2); Thomas (2); Thomas, *et al.* (3); Thomsen (4); Beard and Barnes (3); Beard, *et al.* (7); Tripoli and Beard (5); Stekol and Schmidt (1); Mettel and Slocum (1); Schmitt (1); Zwarenstein (2); Scaglioni (1); Bender (1); Urechia and Retezeanu (1); Reese, *et al.* (1); Kleinschmidt (1); and Mourot (1).

Glycollic acid: Milhorat and Toscani (1).

Glycine with phosphate: Milhorat (6).

Leucine: Degan (2).

Leucyl-glycine: Gradinesco and Degan (5).

Alanine: Beard and Barnes (3); Zwarenstein (2); and Degan (2).

Arginine: Beard and Barnes (3); Shapiro and Zwarenstein (3); Takahashi (1); Abderhalden and Buadze (2); Sekine (1); Kleinschmidt (1); Beard and Boggess (33); Mourot (1); Thompson (1); Gross and Steenbock (1); Crowdle and Sherwin (1); and Degan (3).*

Argininic Acid: Felix and Müller (1); Müller and Bräutigam (2).

Histidine: Mourot (1); Degan (4); Nogami (1); Gon-

* Hyde and Rose (6) administered 1 gm. of arginine per day to a young man and young woman for 6 to 8 weeks. They calculated a possible excess production of creatine amounting to 42 and 56 gm., respectively. There was no increased excretion of either creatine or creatinine. Grant, *et al.* (2) stated that oral administration of arginine for a period of 35 days failed to influence the excretion of creatine or creatinine. In view of the fact that it is now certain that arginine does *increase* creatine formation in the body, it is evident that all of the arginine in the studies of Hyde and Grant must have been destroyed by the arginase of the liver or that the doses of arginine administered were too small. Positive results are always obtained when the amino acids (*e.g.*, arginine) are *injected* into the animal instead of feeding by mouth. Commentating on these and other negative results in regard to arginine as a precursor of creatine, Hunter (1) stated: "So large a body of almost purely negative evidence leads one rather forcibly to suspect that, if creatine is related to arginine at all, its mother substance must not be the free amino acid, but the still combined arginine of the muscle or other protein." This same view had been expressed previously by Rose and by Thomas. The evidence for arginine as a mother substance of creatine at the present time is irrefutable, especially the results of Schoenheimer and his colleagues using their tracer technique. This fact serves anew to lend great weight to the original and extensive studies of Thompson (1) with arginine, and also shows that the positive, though indirect evidence for arginine and other amino acids as precursors of creatine, obtained by many workers in the past, can now be accepted as proving beyond doubt that these amino acids are the most logical precursors of both creatine and creatinine in the body. The views of Hunter, Rose and Thomas that arginine combined in the form of muscle protein may be a precursor of creatine, but that the free amino acid is not, can no longer be accepted. The body makes no distinction between the free or combined arginine for the purposes of creatine formation.

zalez-Rubiera (1); Beard and Barnes (3); Abderhalden and Buadze (2, 3, 4); Masai and Fukutomi (1).

Cystine: Beard and Barnes (3); Masai and Fukutomi (1).

Tyrosine: Degan (4); Nogami (1); Gonzalez-Rubiera (1); Beard and Barnes (3).

Glutamic acid: Beard and Barnes (3); Beard, *et al.* (7).

Aspartic acid: Beard and Barnes (3).

Valine: Beard and Barnes (3).

Caffein, Theobromine and Xanthine: Beard and Pizzolato (15); Koven and Beard (16); and Bachmann, *et al.* (2).

Water: Koven and Beard (16); Beard, *et al.* (22); and Hawk and Fowler (1).

Creatinuria also occurs in the following conditions: after administration of iodoacetate (Milhorat, *et al.* (1)); blockade of the reticuloendothelial system (Mori (1), Terroine and Nataf (1)); after fractures (Cuthbertson, *et al.* (2)); after exposure to X-rays (Miyazakim (1)); after exposure to low temperatures (Milhorat, *et al.* (7), Terroine, *et al.* (2)).

Albanese, *et al.* (1) showed that in 4 subjects fed upon tryptophane-, lysine- or arginine-free diets, there was no change in the excretion of urea, creatinine or ammonia.

Creatinuria due to feeding diets rich in protein has long been known. Some statements of Hunter (1) may be recalled in this connection.

"That a high level of protein intake may under certain conditions be a cause of creatinuria appears to have been fully demonstrated. It is to be remarked, though, that practically all of the evidence which justifies this conclusion is drawn either from young animals, or from those, like women or the subjects of muscular dystrophies, whose muscles approach in a sense the juvenile type. Even in these the extent to which creatine production can be increased by dietary protein seems to be limited. It would seem, therefore, to be by no means certain that the effect

of protein is to be interpreted as proving, under any circumstances, an exogenous source for creatine, in the sense that the latter may arise directly, like urea, from certain precursors in the ingested protein molecule. There may be another explanation of the phenomenon. It may represent merely one phase of that general stimulation of metabolism which is described as the specific dynamic action. An increased *endogenous* production of creatine resulting from such stimulation, might be expected to manifest itself in just the sort of creatinuria that follows ingestion. It would be brought about only by catabolized protein, since protein deposited in the form of new tissue exerts no specific dynamic action (Lusk (1)). It would be at its height, as Denis and Kramer (1) found it to be during the second and third hours after ingestion, when, as Williams, *et al.* (2) have shown, heat production has reached its maximum, but the output of urea is still rising. It would occur most readily in those conditions where the metabolism is already relatively high, as in exophthalmic goiter or in childhood. Such an explanation of the creatinuria following a high protein diet seems, therefore, to be not unworthy of consideration. Protein feeding will increase the output of uric acid: but no one has sought the origin of uric acid directly in the protein of the diet. Lewis, *et al.* (2) and Rose (4) have shown reason to believe that proteins increase the production of endogenous uric acid by virtue of their general property of stimulating all cellular metabolism: it does not seem improbable that they should simultaneously increase the production of endogenous creatine. It is in accord with this suggestion that urinary creatine is found to be increased not only by the amino acid arginine, which Gross and Steenbock (1) take to be a direct precursor, but also, and in an equal degree, by cystine, to which they deny that rôle."

The above reasoning is no longer tenable due to the recent discoveries in the field of creatine and creatinine metabolism. In the first place no distinction can be drawn between the so-called exogenous and endogenous metabolism; second, the author showed in 1927 (3) that creatine formation from the amino acids was not due to their specific dynamic action; third, creatine and creatinine arise during the catabolism of

protein, just as does urea, and in fact urea may well be a precursor of both creatine and creatinine; and fourth, irrefutable evidence has been published by numerous workers to show that the amino acids of the protein molecule, or the individual amino acids themselves, are the natural precursors of creatine and creatinine in the body.

In recent years much additional evidence has been published to show that the feeding of protein will serve to increase both the creatine and creatinine excretion. This will happen, however, only when the animals are fed on diets *rich* in protein. Most recent observers assume that the proteins serve as exogenous sources of creatine and creatinine and this is a confirmation of the results of the earlier literature on the subject (Bollman (1); Burns and Orr (1); Chanutin and Ludewig (4); Christman and Mosier (1); Denis (4); Deuel, *et al.* (8, 9); Eimer (2); Folin (8); Garot (21); Gibson and Martin (1); Harding and Gaebler (1, 2); Harding and Young (3); Klercker (1, 2); Krüger (1); Marples and Levine (1); Levene and Kristeller (1); McCollum (1, 2); McCollum and Hoagland (3); McCollum and Steenbock (4); Palladin and Kratinowa (3); Plimmer, *et al.* (1); Rapinesi (1); Beard and Barnes (3); Beard and Boggess (4); Rose, *et al.* (1, 6); Steenbock and Gross (2); Taylor and Chew (1); Terroine, *et al.* (8-16); Zickelbein (1); and Zwarenstein (2)). An increase in creatine and creatinine in the blood also occurs after feeding protein (Kafieva (1); Myschkis (1); and C. C. Wang, *et al.* (2)).

Much recent work from Terroine's laboratory also shows that creatine is synthesized from the tissue proteins (Terroine, *et al.* (3, 5); Terroine (6); and Pariset (4)). Zamagi (1) showed that in fasting rats the excretion of creatine was reduced while that of creatinine was increased. Daft, *et al.* (1) observed that the production of a sterile abscess in a dog caused similar increases in creatine and nitrogen excretion.

Injection of dog plasma into a protein depleted dog likewise caused an increase in the excretion of creatine, ammonia and urea.

Beard and Barnes (3) showed in 1925 that the feeding of gelatin and casein to man would result in increased creatinine excretion (Table 6). Borsook, *et al.* (5) obtained glycozamine from gelatin and Dill and Horvath (1) noticed an increased excretion of creatine in 2 out of 4 men after ingesting 60 gm. of gelatin daily. After cessation of the gelatin ingestion increases in both creatine and creatinine excretion occurred in all 4 subjects. This was evidently due to a storage of both creatine and creatinine from the gelatin feeding.

It has long been known that any interference with normal liver function will cause creatinuria. Daft, *et al.* (2) showed that chloroform anesthesia produced a large increase in creatine excretion due to liver injury. Koven and Beard (29) observed increases in creatine excretion after administering chloroform or ether to the rat. Urethane increased creatine, but not creatinine excretion, while amytal increased the excretion of both creatine and creatinine. L. L. Miller (1) observed a large creatinuria in the dog after anaphylactic shock produced by the injection of horse serum. Masuda (1) stated that the liver was unable to convert creatine into creatinine. Norboru (1) showed that only a small part of creatine fed to rabbits was excreted as creatine and creatinine. Parfentjev and Perlzweig (1) state that mouse urine contains 45 per cent of its total creatinine excretion in the form of creatine.

In experimental and clinical studies on creatinuria the investigators usually feed a creatine-free diet in order that no creatine from exogenous sources will interfere with the results of the study. The writer thinks that this should not be done. It is an established fact that the amino acids, such as glycine, *will not form creatine* if diets low in protein are

ingested. The protein requirements of the tissues must first be met, otherwise the ingested glycine will evidently be utilized for the formation of tissue proteins and not for the formation of creatine. Therefore a diet containing meat, or other adequate protein should be ingested with glycine, if the best results are to be obtained. The same applies to the clinical improvement to be expected in the myopathy patient treated with glycine. It is, therefore, possible that the negative results of Hyde (1, 6, 7) who fed meat-free diets to her subjects may be explained on this basis.

The creatinuria of carbohydrate deprivation and inanition will be discussed in Chapter XIV.

In his extensive studies E. Wang (1) showed that there was no relation between the endogenous and exogenous creatinuria. This means that endogenous and exogenous creatine are metabolized in different ways. Also no increase in creatinine elimination occurred after creatine administration. These views are confirmatory of those expressed in Chapter VI on the origin of creatine, showing that exogenous and endogenous creatine and creatinine are metabolized in a different manner.

Creatinine Phosphoric Acid. Zeile and Hildegard (1) stated that an old preparation of calcium isocreatine phosphate was found to have lost water spontaneously to form calcium creatinine phosphoric acid. In these compounds the phosphorus is attached to the nitrogen atom outside the ring. Creatine phosphoric acid dichloride + aniline = creatinine phosphoric acid dianilide, M. P. 224–6° C. with decomposition. Later Zeile and Meyer (2) showed that the above compound was creatinine phosphate, the calcium salt of which shows dimorphism and upon alkaline hydrolysis yields creatine phosphate. The smooth hydrolysis of creatinine phosphate to creatine phosphate indicates that in creatine phos-

phate the PO_4 is either attached to NH_2 or NH , if they preexist. Creatinine phosphate has two buffer regions, pK 7.38 and 3.41 respectively. Creatine phosphate has pK 2.81 and 2.44. On treatment of creatine phosphate with dry HCl no creatinine phosphate is obtained, but methyl hydantoin phosphate is formed.

Geiger (1) attempted to separate the glycolytic enzyme system from brain cells and its coenzyme. Cozymase, adenosine triphosphate, apozymase, glutathione and yeast juice heated in boiling water for 3 minutes and creatinine phosphate were used alone and together as activators. Creatinine phosphate was stated to be the coenzyme of the glucose splitting system.

Baeyer and Muralt (1) measured the typical color curves and extinction ($\gamma = 570\text{m}\mu$) of frogs' sartorius muscle in the spectrophotometer. During anaerobic fatigue there was a fall in the extinction parallel to the decrease in creatinine phosphoric acid * followed by a rise in the extinction parallel to the lactic acid production.

From the above evidence it seems possible that creatinine phosphoric acid has been identified and its future rôle in biological reactions will be awaited with much interest.

Creatine Tolerance. Since the introduction of amino acetic acid ingestion in the myopathies, the creatine tolerance test has become a routine procedure. Since creatinuria occurs in a number of different conditions not involving the muscular system, these conditions must be ruled out before the creatine excretion can have any diagnostic value. It is also very un-

*In the abstract of the papers of Baeyer and Muralt, which was the only source of information available to the writer, *creatinine* phosphate was the term used. However, Doctor Lipmann stated that, after reading the original papers of these authors, that *creatine* phosphate, rather than creatinine phosphate, was the term used.

likely that creatinuria alone would be used in this connection, since the myopathies are, in general, easily recognized by physical diagnosis and other means.

The amount of creatine excreted depends upon the amount administered. This may only be about 1 or 2 per cent of the amount of creatine stored in the tissues. One of the chief criticisms that may be raised against the creatine tolerance test is that large variations, as mentioned above, in creatine retention and excretion occur in normal individuals, in children (Wilkins, *et al.* (1)), and in myopathic individuals after creatine ingestion. Milhorat and Wolff (8) stated that neither the creatine output nor the creatine tolerance associated with muscular wasting subsequent to diseases of the nervous system appears to be a valuable index of the amount of muscular involvement. Netolitzky and Pilcher (1) observed that an improvement in creatine tolerance is no measure of an increase in function of the dystrophic musculature. Sohval, *et al.* (1) stated that the creatine tolerance test is not constantly positive or pathognomonic in patients with Grave's disease. Thomas (4) and Wilkins, *et al.* (1) both stated that the creatine tolerance test in children had no diagnostic value. E. Wang (1) and Espersen and Thomsen (2) also expressed the view that the creatine tolerance test was of little diagnostic value. Another fact is that ingested creatine can be oxidized by the enzyme *creatine oxidase* present in the tissues (Beard (37)). Hence the differences in the degree of storage or oxidation of creatine after its ingestion can have little physiological significance. Since creatine is not transformed into creatinine, the creatine administered in the creatine tolerance test is simply oxidized into other unknown substances, so the test should be renamed the "creatine oxidation test" rather than the creatine tolerance test.

It is well known that the defect in the myopathy patient is not one of creatine or glycine synthesis, but a defect in the

utilization of creatine produced in the body from its precursors. Since administered creatine is largely excreted unchanged and the amount that is retained is not utilized to any great extent, it is evident that administered creatine can serve no useful function in this type of patient. Creatine, however, formed in the body from its amino acid precursors, is utilized by the patient. But this again depends upon the amount of functional muscle tissue remaining in the patient, the amount of available phosphate, the length of time the dystrophy has been present, and most important, the *length of time that glycine ingestion is administered*. The ingestion of phosphate with creatine might result in increased retention of creatine which may then have some physiological significance.

Results of recent research show that the nitrogenous products excreted into the urine are not entirely waste products. Ammonia, creatinine, uric acid, and urea can each be transformed into creatine and creatinine. Even carbon dioxide, in addition to its stimulating effect upon the respiratory center, can be transformed into urea and into glycogen. These are additional illustrations of the many possible pathways a given substance may follow in metabolism.

CHAPTER IX

THE BIOLOGICAL RELATION BETWEEN ADMINISTERED CREATINE AND CREATININE

EVER SINCE the discovery of these substances many observers have believed that creatinine arises by the dehydration of creatine in the body. Before 1906 experimental evidence for this transformation was not considered necessary to prove it. It is well known that the equilibrium between these two substances depends upon the pH, which in the tissues is slightly alkaline, and this would tend to shift the equilibrium from creatinine to creatine. Furthermore the water content of the tissues also favors this change. Brazda, *et al.* (20) observed that four times as much creatinine was transformed into creatine, as compared to the reverse process, at pH ranges from 6.7 to 7.6, in buffered aqueous solutions of creatine and creatinine, incubated at 37° C. for two weeks. Muscle contains a factor which, at different pH ranges, causes a disappearance of about 25 per cent of the creatinine originally present in the solution.

The studies of Benedict and Osterberg (1) and Chanutin (3) are usually cited to prove that creatine is changed into creatinine in the body. But Benedict and Osterberg stated that the change was a slow one at best, only about one-third of creatine being changed into creatinine, while the recent

studies of Borsook and Dubnoff (2, 3, 4) show that creatine formation is rapid when kidney and liver slices are incubated with arginine, glycine and methionine. The large doses of creatine administered to Chanutin's subjects can be considered unphysiological since these amounts of creatine would never arise in the body during the course of normal protein metabolism. Bollmann (1) stated that the creatine-creatinine transformation was much increased when creatine and a high protein diet were fed together.

In 1913 Myers and Fine (15) studied the effect of administering creatine and creatinine upon the creatine content of rabbit muscle and urine. The injection of creatine raised the creatine content of the muscle slightly and caused an increase of about 2 or 3 per cent in creatinine excretion. In regard to the injection of creatinine Myers and Fine stated, "The increase in the creatine content of the muscles is, if anything, more pronounced after the administration of creatinine than of creatine. . . . That creatinine exerts this influence, is in harmony with the view that the reaction between these two substances is reversible. This is further borne out by the observations of other workers of an excretion of creatine following the administration of creatinine. . . . It would seem then, that by the administration of either creatine or creatinine the concentration of muscle creatine may be raised by about 5 or 6 per cent above the values ordinarily obtained. That the extra creatine is quite firmly held, is indicated by the fact that the concentration of the creatine in the muscle is practically the same whether the rabbit is killed one day or four days after the last injection. . . . The experimental data in the case of creatinine seem to indicate that the 20 per cent which is not excreted in the urine may be completely stored up in the muscle as creatine."

Recently Bloch and Schoenheimer (5) fed creatine containing N^{15} to rats. Creatinine containing N^{15} was isolated

from the urine. This is direct proof that creatine is changed into creatinine, but the writer has criticized their negative studies with creatinine (Beard and Pizzolato (26)). The New York investigators stated that about 50 per cent of creatinine containing N^{15} is excreted as such in the urine and that creatine isolated from the muscles did not contain any of the isotope. From these results they concluded that the biological transformation of creatine into creatinine was irreversible. It is entirely possible that the other 50 per cent of the creatinine containing N^{15} which was not accounted for might have stimulated creatine formation and excretion. The increase in urinary creatinine was 75 per cent of that fed, showing a possible retention of some creatinine, which again could have stimulated creatine formation and excretion.

The extra amount of creatine formed, if any, was *not* determined colorimetrically. The isotope is only a "qualitative tag" so to speak, and in order for their conclusions to be valid it would be necessary to actually determine the total amount of creatine in both muscle and urine. This would then shift the emphasis from the isotope to creatine itself, since it is the physiological formation of the latter that is of most interest. Furthermore, we have shown that about 25 per cent of pure creatinine in solution at 37° C. disappears in the course of 2 weeks (20) (37) and it is possible that some of the isotopic creatinine was not traced in their studies. For instance, glycine containing N^{15} not only is transformed into creatine containing N^{15} , but also into the glycine of proteins and glutathione, etc. Hevesy (2), in discussing radioactive indicators, stated that, in cases of studies on radioactive phosphorus, the determination of the P^{32} and P^{31} content of the sample should both be made.

In our extensive studies with many hundreds of rats, increases in muscle creatine last for only 2 days after the precursor of creatine is fed or injected. From this time on the

extra creatine is excreted as such into the urine. It is, therefore, evident that at the end of 6 days after creatinine feeding they should not expect to find any extra creatine containing the isotope in the muscles of their animals. Neither did Bloch and Schoenheimer study urinary creatine containing N^{15} , so they are not in a position to state that the creatinine was not transformed into creatine which was later excreted into the urine.

Their dose of creatinine isotope used was not stated. We have found that it requires from 10 to 20 mg. of injected creatinine to cause an increase of 15 to 20 per cent in muscle creatine accompanied by an increase of 48 mg. of extra creatine excretion in the urine. Their 6 day experimental period does not, therefore, allow sufficient time to obtain all of the creatine formation and excretion after creatinine is injected. In our studies, it required from 2 to 3 weeks for this purpose after the injection of a given amount of creatinine or water. We have shown that the creatinine \rightarrow creatine change is also under the influence of the posterior pituitary hormones (Beard and Pizzolato (26)). These hormones were not studied by Bloch and Schoenheimer. Their conclusions were drawn from results with only 3 animals. The large variations in creatine-creatinine formation and excretion is, therefore, not appreciated by the New York workers. While their positive results showing the transformation of creatine into creatinine cannot be denied, nevertheless their negative results with creatinine are open to serious question since we have shown with over 80 young rats that the creatine-creatinine transformation does *not* occur while the reverse process did occur (Beard, *et al.* (15, 17, 18, 22, 23, 24, 26, 37)).

Hyde (7) has recently studied the effect of administering creatine on the creatine and creatinine excretion in 13 men and women. The average retained creatine, excreted as creatinine, increased about 9.5 per cent over the control value

when 1 gm. of creatine was ingested daily by her subjects. If about a 5 per cent error is allowed for matching the creatinine color in the urine with the visual colorimeter, it is seen that only a slight transformation of the retained creatine was transformed into creatinine and excreted. The excretion of extra creatinine under these conditions varied from 0 to 20 per cent in the different subjects. If this evidence is accepted as proving a transformation of exogenous creatine into creatinine in man it is still seen that the magnitude of the change in no way compares to the magnitude of the change from creatinine into creatine to be discussed later. It was also shown that the ability to transform ingested creatine into creatinine varied irrespective of age, sex, muscle development, creatinine coefficient, or degree of creatinuria on a creatine-free diet. The creatinuria of adults, old or young, could not be explained by an inability to dehydrate exogenous creatine. No correlation was shown between, on the one hand, the capacity to retain exogenous creatine, and, on the other hand, the extent to which the retained creatine was dehydrated, the degree of creatinuria on a creatine-free diet, or the creatinine coefficient. Creatine and inorganic phosphorus retentions were not related. It should not be forgotten that these subjects ingested, for the most part, meat-free diets. Whether the ingestion of meat by her subjects would have altered the results is still an open question.

Folin (8) was the first to deny the biological transformation of creatine into creatinine (Table 17). Similar evidence was soon published by Klerecker (1, 2), Lefmann (1), and many others (*Cf.* Hunter (1)). In more recent years Terroine and Boy (10), Zickelbein (1), K. Thomas, *et al.* (3), Daniels and Hejinian (1), Catherwood and Stearns (1), and E. Wang (1) have also questioned the transformation of creatine into creatinine.

Another fact pointing to non-relation of creatine and creatinine is that the former occurs in much greater concentration in the tissues than the latter. If creatine were transformed into creatinine some of the latter should be retained for a time in the tissues before being excreted. Creatinine occurs in traces in the tissues only and is known to be readily and rapidly excreted by the kidneys. Many experimental procedures will increase or decrease the creatine excretion while that of creatinine may remain fairly constant under these conditions.

In all of our studies, the experimental conditions were very favorable for the creatine-creatinine transformation. In fact, in some cases the injection of creatine actually caused a retention of creatinine. Another fact should be remembered here: that the metabolism of *administered* creatine and creatinine is different from creatine and creatinine formed during the normal course of protein metabolism in the body. We are discussing the effects of *administered creatine and creatinine*, therefore no studies up to the present give information on whether or not the creatine and creatinine of the tissues have any biological relation to each other. The following scheme, however, can be offered for the biological relation of administered creatine and creatinine in the body:

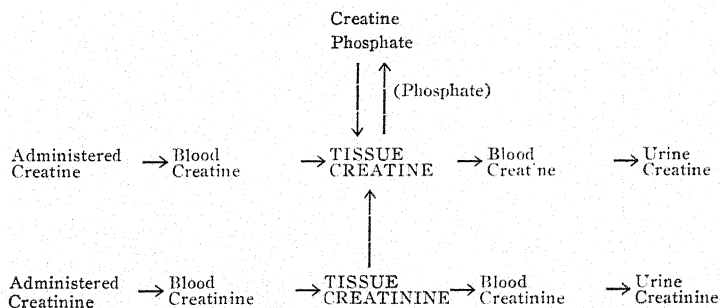


FIG. I

Almquist, *et al.* (3) studied creatine formation in the chick. Glycine, arginine, glycine plus arginine, gelatin, glycoxyamine, creatine and creatinine in the diet of the chick lead to increased muscle creatine content and increased rate of growth as compared to that of the controls. When creatine precursors are absent from the diet, the content of muscle creatine is abnormally low and muscular attenuation and profound muscular weakness occur.

Of particular interest in this connection was the effect of the transformation of creatinine into creatine in the chick. The control creatine in the breast muscle was 3.74 mg. per gm. This value after feeding the following compounds was: glycoxyamine, 5; creatine hydrate, 4.83; creatinine, 5.19; arginine-HCl, 4.04. It is, therefore, seen that creatinine was the most effective creatine former of all the compounds studied, since it increased the creatine content by 39 per cent above that of the controls.

The results on creatinine formation were equally interesting. The control creatinine content of the breast muscle was 0.06 mg. per gm. This value after feeding the following compounds was: glycoxyamine, 0.10; *creatine hydrate*, 0.09; creatinine, 0.16; arginine-HCl, 0.06.

It is seen that there might have been a very slight transformation of creatine into creatinine in these studies, but it is very difficult to believe that the difference of 0.03 mg. of creatinine between the control and the creatine fed animals is significant. The absolute amount of creatine increase from creatinine was 1.45 mg. per gm. of breast muscle while this absolute increase when creatine was fed in creatinine formation was *only* 0.03 mg. It is, therefore, possible that, in the chick the transformation of creatinine into creatine would be more likely to occur than that of the reverse process.

Zeile and Hildegard (1) showed that creatinine phosphate

was easily transformed into creatine phosphate while hydrolysis of the latter did not yield creatinine phosphate, but methyl hydantoin phosphate instead.

The writer (37) has recently completed further work to illustrate the transformation of creatinine into creatine *in vitro*. In our earlier study (Brazda, *et al.* (20)) it was observed that about 25 per cent of the creatinine present in the solution disappeared when the solution was incubated in the phosphate buffer for 2 weeks at 37° C. It is reasonable to believe that this creatinine has been oxidized. To several flasks, each containing 50 mg. of creatinine in 50 cc. of the phosphate buffer solution, different tissues and hormones were added and oxygen bubbled through the mixture for 24 hours. The hormones used were thyroxine, pitressin, and pitocin and their addition to the flasks had no effect upon the oxidation and hydration of creatinine.

Under these conditions creatinine was completely oxidized in 2 days. In spite of this the creatine formation from creatinine from highest to lowest in the presence of the following tissues was as follows: lung, kidney, liver, pancreas, and muscle. In another study no oxygen was admitted. Creatine formation from creatinine at the end of the first 10 days' incubation from highest to lowest was as follows: (lung, muscle, stomach,) liver, pancreas, kidney, and spleen. The addition of 0.15 M cyanide, or boiling the tissues before their addition to the bottles, destroyed the effect of the tissues on these transformations.

The following names were suggested by the writer for the enzymes causing these changes, *creatinine oxidase*, *creatinine hydrase*, and *creatine oxidase*. In another study using 100 cc. of buffer solution containing 100 mg. of creatinine the results shown below were obtained. These results from highest to lowest may be summarized as follows:

Creatinine oxidase → heart, intestine, liver, blood, stomach, (5 days' incubation) muscle, kidney, (brain, lung, and liver.)

Creatinine oxidase → kidney, heart, liver, muscle, intestine, (10 days' incubation) blood, stomach, lung, testes, and brain.

Creatinine hydase → intestine, kidney, muscle, heart, testes, stomach, blood, brain, and liver. (5 days' incubation)

Creatine oxidase → intestine, kidney, heart, stomach, (10 days' incubation) muscle, testes, lung, blood, brain, and liver.

The color of the Jaffé reaction with alkaline picrate in the total creatinine flasks was destroyed by the specific creatinine enzyme of Miller and Dubos. It is of interest in this connection that Baumann and Ingvaldsen (1) showed that creatine could be oxidized to methyl guanidine and oxalic acid.

The results of our study offer strong evidence for the views of the writer that creatinine can be changed into creatine *in situ* in the tissues and show that the theory of Bloch and Schoenheimer (5) that creatine cannot be formed from creatinine is untenable. Gottleib and Stangassinger (1) and Stangassinger (2) in 1907 stated that liver, muscle, spleen, lung, and blood contained enzymes that destroyed creatine and creatinine and named these enzymes "creatase" and "creatinase", respectively. They also stated that in these tissues there may be a new formation of creatine from unknown sources. Hunter (1), however, in his monograph on Creatine and Creatinine, in discussing these studies, stated: "those results in particular which implied an extensive destruction of creatine or creatinine during autolysis have been completely discredited. "Creatase" and "Creatinase" have, therefore, now practically disappeared from the literature." However, our results reported above leave no doubt but that there is an extensive destruction of creatinine as well as creatine

formation from creatinine in the presence of various tissues.

Baker and Miller (1) observed that the concentration of creatine and creatinine were roughly proportional in different tissues of the rat indicating an interrelationship of these two compounds in metabolism. The interesting observation was made that the concentration of creatinine in the spleen, liver, lung, and pancreas, was much lower than in an ultra-filtrate of plasma and this indicated that these tissues are concerned with the transformation of creatinine into creatine and other compounds.

Horvath and Dill (3) allowed urines preserved with toluene to stand for different lengths of time at room temperature. There was a transformation of creatinine into creatine under these conditions. This transformation also occurs at 4° C. provided a longer time is allowed for the reaction to occur.

The author (37) also incubated solutions of creatine with the different tissues (Table 24). The transformation of creatine into creatinine *was the same* in the flasks containing the tissues as it was in the control flasks. It is, therefore, evident that these tissues do not contain a *creatine anhydrase* and that the creatine-creatinine transformation did not occur in these studies. Hammett (1) studied this reaction in 1922. He was likewise unable to discover an enzyme in muscle tissue that catalyzed the reaction of creatine into creatinine.

Beard and Jacob (17) administered both creatine and creatinine to rats and to men to study their influence upon the creatine-creatinine excretion in the urine. The results obtained were listed in Tables 18 to 21 and discussed in Chapter VIII. When creatinine was injected into young rats increases in muscle creatine from 19 to 41 per cent above normal were observed (Table 25) and a stimulation of both creatine and creatinine excretion occurred (Table 20). The same was true after creatinine ingestion by man (Table 21). Ingestion of

TABLE 24
CREATINE-CREATININE TRANSFORMATION *IN VITRO*
(After Beard (37))

		DAYS AFTER INCUBATION AT 37° C.					
		5		10		20	
		Creat- inine, mg.	as Creat- inine, mg.	Creat- inine, mg.	as Creat- inine, mg.	Creat- inine, mg.	as Creat- inine, mg.
Creatine,	to Creatine,						
mg.	gm.						
100		16.8	83.0	21.5	73.0	11.5	65.0
100		15.4	83.0	21.5	73.0	12.0	68.0
100		16.7	83.0	26.5	73.0	12.5	68.0
100		19.5	80.0	17.5	64.0	10.5	65.0
	Liver, 6	2.0	0.2	0.0	0.0	0.0	0.0
100	Intestine, 8	19.5	83.0	12.5	64.0	10.5	65.0
	Intestine, 8	1.3	2.6	0.0	0.6	0.0	0.0
100	Muscle, 4	18.0	92.0	21.5	88.5	10.5	83.0
	Muscle, 4	3.0	6.0	1.0	2.2	0.0	0.0
100	Kidney, 4	18.0	89.0	16.5	73.0	10.5	72.0
	Kidney, 4	3.2	2.3	0.0	0.0	0.0	0.0
100	Lung, 4	16.8	85.0	15.0	72.0	10.7	72.0
	Lung, 4	2.0	1.0	0.0	0.0	0.0	0.0
100	Heart, 4	16.8	87.0	11.5	72.0	10.4	72.0
	Heart, 4	1.4	1.7	0.0	0.3	0.0	0.0
100	Testes, 4	16.0	88.2	19.0	73.0	10.5	76.0
	Testes, 4	1.6	3.0	0.0	1.1	0.0	0.0
100	Blood (7 cc.)	18.0	75.0	16.5	73.0	10.2	73.0
	Blood (7 cc.)	1.9	2.6	0.0	0.0	0.0	0.0
100	Stomach, 4	16.0	75.0	12.5	62.0	10.5	65.0
	Stomach, 4	2.0	1.5	0.0	0.0	0.0	0.0

creatinine by the rat and man did not increase creatinine excretion, but caused its retention instead (Tables 18 and 19). Injected creatinine did not increase the creatinine content of muscle tissue.

If it were true that creatine is transformed into creatinine in the body then an increase in creatinine excretion should be accompanied by an increase in creatine formation. But it is well known that, under the usual conditions of metabolism, the excretion of creatine and creatinine are usually independent of each other and they do not necessarily vary together. In starvation there is creatinuria with a normal creatinine excretion. In fever the excretion of creatinine may rise without accompanying creatinuria. In several of our studies creatinuria occurred with a normal creatinine output.

In some of our studies mentioned above creatinine re-

TABLE 25
EFFECT OF PARENTERAL INJECTION OF CREATININE
UPON CREATINE-CREATININE CONTENT OF
MUSCLE TISSUE
(After Beard and Jacob (17))

No. of Animals	Creatinine Injected, mg.	Average Muscle Creatine, Per Cent	Average Increase in Muscle Creatine, Per Cent	Average Muscle Creatinine, Per Cent	Duration of Ex- periment, Days
48	Control	0.42		0.02	2
6	Control	0.42		0.02	2
2	10	0.50	19.0	0.01	2
2	20	0.49	16.6	0.01	2
2	30	0.55	30.9	0.01	2
2	40	0.51	21.4	0.01	2
2	50	0.50	19.0	0.01	2
2	60	0.48	14.3	0.02	2
2	75	0.54	28.6	0.03	2
2	100	0.56	33.3	0.03	2
2	150	0.59	40.5	0.03	2
2	200	0.53	26.2	0.03	2

tention occurred with creatinuria. Either some of this creatine escapes conversion to creatinine or, more probably, the retained creatinine is quantitatively transformed into creatine and excreted. In Chapter XI further data on the transformation of creatinine into creatine under the influence of the posterior pituitary hormones will be given.

From the recent results in creatine-creatinine metabolism the author is of the opinion that there is no biological relation between *administered creatine* and creatinine. On the other hand there is a definite biological relation between *administered creatinine* and creatine. This leaves the question of the biological relation between *body creatine* and creatinine unanswered, since there is no method of studying this relationship at the present time, except by the use of the tracer technique. Further results with this technique should be of much interest in this connection provided the proper experimental conditions are employed in the tests.

CHAPTER X

RELATION OF CREATINE-CREATININE METABOLISM TO METABOLISM OF WATER

IF IT is true that creatinine can be hydrated to creatine in the body then it is easy to visualize the rôle of water or physiological saline in this reaction. This view receives additional support from the analogous findings of Schoenheimer and Rittenberg (11) that mice can synthesize alternate hydrogen atoms of cholesterol from the heavy water fed to them. Hawk and Fowler (1) many years ago reported an extra excretion of 1.5 gm. of creatine when their subject drank 3 liters of water daily with his meals.

Beard, *et al.* (22) studied the effect of injecting water and physiological saline into rats and their ingestion by man upon the creatine content of rats' muscle and upon creatine-creatinine excretion in both the rat and man. The study was divided into 4 parts: (1) a comparison of the effect of injection or ingestion of water and physiological saline upon creatine-creatinine excretion in the rat and man; (2) the effect of injected saline with and without creatinine upon creatine-creatinine excretion; (3) the effect of injected saline upon tissue creatine; and (4) the effect of feeding sodium bicarbonate and disodium phosphate upon creatine-creatinine excretion.

The injection of 1 cc. of water into the rat caused an

extra excretion of 75 mg. of creatine within 22 days after injection. In previous studies the average amount of extra creatine excreted under these conditions was 35 to 40 mg. The injection of 2 cc. of water caused an extra excretion of 113 mg. of creatine and 37 mg. of creatinine. The injection of 6 cc. of water had no greater effect than the injection of 2 cc. In a repeat study with the same animals under identical conditions the extra excretion of creatine was only 10 mg. In another study with these same animals from 1 to 4 cc. of physiological saline was injected. This resulted in an average increase of 65 mg. of creatine excretion which was accompanied by an average *retention* of 40 mg. of creatinine. A possible explanation of the effect of water and saline upon creatine excretion is furnished by the results of Hevesy and Hofer (1) who showed that a molecule of heavy water remained in the body for 2 weeks.

When massive doses (144 mg.) of saline were injected into 8 rats in 8 cc. of water daily for 2 days there was an increased excretion of 147 mg. of creatine accompanied by a retention of 47 mg. of creatinine. In the same rats after castration the extra creatine excretion was 101 mg. without any effect upon the creatinine retention or excretion. Most of the extra creatine was excreted within 8 days after saline injection. A second study on the effect of water and salt for the next 8 days showed only small extra excretions of 22 and 20 mg. of creatine. It is, therefore, possible that the retention of water under the influence of saline was responsible for the creatinine to creatine transformation. It is also evident that body creatinine was transformed into creatine in the presence of the gonads and that in their absence saline caused only a stimulation of creatine excretion without influencing the creatinine excretion.

The data listed in Table 26 show the effect of water and saline ingestion on creatine-creatinine excretion in man.

TABLE 26

EFFECT OF WATER AND SALT INGESTION UPON URINE
VOLUME AND CREATINE-CREATININE EXCRETION
IN MAN(After Beard, *et al.* (22))

	CONTROL PERIOD (16 DAYS)				EXPERIMENTAL PERIOD (28 DAYS)				
	TOTAL EXCRETION				TOTAL EXCRETION				
	OF EXTRA				OF EXTRA				
	Creatine				Creatine				
	Total Water Intake, liter	Total Urine Volume, liter	(as Creat- inine), gm.	Creat- inine, gm.	Total Water Intake, liter	Total Salt Intake, gm.	Total Urine Volume, liter	(as Creat- inine), gm.	Creat- inine gm.
Subject									
P.P.	36.5	40.6	3.9	2.3	48.7	10	50.5	5.7	3.8
H.H.B.	24.2	24.6	3.2	10.2	46.4	20	44.8	11.2	5.3

Subject PP ingested 5 liters of water during the third 2-day period. This was 2 liters above his usual daily water intake. Four liters more urine were excreted than water ingested during the next 16 days. The increase in extra creatine and creatinine excretion was 3.9 and 2.3 gm., respectively. In the next period this same subject ingested 10 gm. of NaCl in 8 liters of water during the third 2-day period. The subject was practically in water balance during the next 28 days. The increase in extra creatine and creatinine excretion was 5.7 and 3.8 gm., respectively. This was almost double his excretion of creatine and creatinine from the ingestion of the same amount of water alone. Subject HHB ingested 5 liters of water during his third 2-day period. He was in water balance during the next 16 days. The extra creatine and creatinine excretion was 3.2 and 10.2 gm., respectively. During the next 2-day period he ingested 20 gm. of NaCl in 6 liters of water. During the next 28 days he ingested 46.3 liters of water and excreted 44.8 liters of urine. A total retention of about 1.5 liters of water occurred during this time. The extra creatine excretion was 11.2 gm. and creatinine excretion of 5.3 gm. as compared to their excretion after the injection of the same amount of water alone. Even though both subjects were essentially in water balance the sudden increase in water and salt intake had a pronounced effect upon creatine and creatinine excretion.

These results show the importance, at least in studies of creatine and creatinine excretion, of knowing the water and salt content of the diet. Also the effect of the fluid medium used to inject a substance should be known, and this effect should be subtracted from that obtained when a given substance is injected in the same amount of fluid.

TABLE 27
EFFECT OF PARENTERAL INJECTION OF PHYSIOLOGICAL
SALINE AND CREATININE UPON (A) CREATININE-
CREATINE AND (B) CREATINE-CREATININE
EXCRETION IN RATS. EXPERIMENTAL
PERIOD, 20 DAYS
(After Beard, *et al.* (22))

Rats, no.	Saline Injected, cc.	CREATININE			Extra Creatine (as Creatinine) Excreted, mg.	
		Injected, mg.	Extra Excreted, mg.	Retained, mg.		
(a) Simultaneous and separate administration, normal rats						
2	2	—	—	—	32.0}	61
2	1	20	—	20	(tr) ¹ 29.6}	
3	2	20	40	—	(s) ² 76.0	
2	4	—	23	—	56.0}	79
2	1	40	25	15	(tr) 23.0}	
3	4	40	51	—	(s) 75.0	
2	6	—	—	—	53.0}	81
2	1	60	29	31	(tr) 28.8}	
3	6	60	35	25	(s) 67.0	
2	8	—	25	—	53.6}	83
2	1	80	40	40	(tr) 30.0}	
3	8	80	7	73	(s) 67.0	
2	10	—	46	—	39.0}	79
2	1	100	55	45	(tr) 40.0}	
3	10	100	117	—	(s) 101.0	
2	2	—	—	—	32.0}	97
2	2	150	110	40	(tr) 65.0}	
3	10	150	120	30	(s) 92.0	
2	2	—	—	—	32.0}	102
2	2	200	91	109	(tr) 70.0}	
3	10	200	167	33	(s) 99.0	

Av. creatine excretion, salt and creatinine together, 82.4 mg.;
separately, 83.3 mg.

¹ tr = transformation of creatinine to creatine.

² s = stimulation of creatine excretion.

TABLE 27 (Continued)

EFFECT OF PARENTERAL INJECTION OF PHYSIOLOGICAL
SALINE AND CREATININE UPON (A) CREATININE-
CREATINE AND (B) CREATINE-CREATININE
EXCRETION IN RATS, EXPERIMENTAL
PERIOD, 20 DAYS
(After Beard, *et al.* (22))

Rats, no.	Saline Injected, cc.	CREATININE			Extra Creatine (as Creatinine) Excreted, mg.
		Injected, mg.	Extra Excreted, mg.	Retained, mg.	
(b) Castrate rats					
2	2		None		33
	2	20	None	20	43
2	4		None		51
	4	40	9	31	44
2	6		None		39
	6	60	18	42	41
2	8		14		59
	8	80	40	40	69
2	10		35		49
	10	100	76	24	56
Av. creatine excretion, salt and creatinine together, 50.6 mg. ; with salt alone, 46.2 mg.					

Simultaneous injection of saline and exogenous creatinine into rats doubled the average creatine excretion usually found following the injection of the same amounts of saline or creatinine separately (Table 27). The creatinuria in these same rats after castration under these conditions was due to a stimulating effect of water or saline upon creatine excretion. The presence of the gonads is again shown to be necessary for the transformation of exogenous creatinine into creatine. The retained exogenous creatinine which was injected in 1 or 2 cc. of saline was, on the average, quantitatively transformed into creatine. These same doses of creatinine injected in 4 to 10 cc. of saline stimulated creatine excretion. The injection of water or saline did not increase

the creatine content of the tissues except in a few cases in the heart.

Increases in muscle creatine were directly proportional to the total solid content of the muscles in 3-day water starved rats. In several other studies with the hormones the increased creatinuria was due to the water content of the preparations injected (16, 23, 25).

It is of interest in this connection that Orr and Rumold (1) showed that the administration of sodium chloride to dogs with pyloric obstruction leads to greater increases in non-purine nitrogen and creatine in the blood than did those receiving water alone. Ide and Hongo (1) showed that the total creatinine of the gastric juice rises and falls with its chloride content.

The feeding of sodium bicarbonate or disodium phosphate to rats greatly increased the creatinine \rightarrow creatine transformation (Beard, *et al.* (22)). Keith and Osterberg (2) and Mason and Hallbaum (1) have shown that alkalosis increases the water content of the tissues. Morgulis and Osherhoff (1) observed a large increase in sodium, chloride, and calcium. Morgulis and Spencer (2) noticed a marked diuresis and increase in creatine and phosphate excretion in dystrophic rabbit muscle. Fenn and Goettsch (1) also observed considerable increases in extracellular water in nutritional muscular dystrophy (*Cf.* Chapter XVI). As stated above Hawk and Fowler (1) many years ago reported an extra excretion of 1.5 gm. of creatine when their subject drank 3 liters of water daily with his meals. Browne, *et al.* (1) showed that the non-adapted animal, just as the animal exhausted by continuous exposure, responds to damaging agents, such as cold, excessive muscular exercise or toxic doses of formaldehyde, with a decrease in water and chloride excretion followed by marked increases in creatine excretion. The adapted animal showed the reverse changes. Cutting,

et al. (1) stated that, after massive infusions of physiological saline, the muscles, spleen and skin store water and saline in proportion to make a 1 per cent solution. McCance and Widdowson (1) observed that severe salt deficiency produced in man by sweating, was accompanied by a fall in the creatinine, sucrose and urea clearances. Titone (1) reported that in cases of intestinal obstruction, as well as nephritis in man, in the absence of irreparable kidney damage, extremely high creatinine values (15–30 mg./per cent) are observed.

Norris and Wieser (1) reported that there is an increased rate of excretion of creatinine, uric acid and chlorides after exercise. Krüger (2) showed that after strenuous exercise there is no increased excretion of creatinine, but there is in creatine excretion (*Cf.* Haldi and Bachmann (1)). Shafer and Skow (1) reported that fatigued or myopathic muscles take up additional water. Since creatinine may be transformed into creatine in the presence of extra water in the tissues, it is reasonable to believe that some of the creatinuria observed after severe exercise (or in the urine of the myopathy patient) may have had its origin in this creatinine of the tissues. It is also possible that some of the creatinuria of nutritional muscular dystrophy (Fenn and Goettsch (1), Goettsch and Brown (2), Knowlton, *et al.* (1) and others) and congestive heart failure (Herrmann (1)), may be explained on this basis, since there is an increase in water in the former and of interstitial fluids in the latter condition (*Cf.* Chapters XVI and XVIII).

The creatinine-creatine transformation is also under the influence of some of the hormones. Beard, *et al.* (23, 24, 26), Pizzolato and Beard (18), Koven and Beard (19), and Koven, *et al.* (25) attributed the creatinuria to water and salt retention in these studies after the injection of several different hormones (*Cf.* Chapter XIII). O. W. Smith (1) is

TABLE 28

EFFECT OF PARENTERAL INJECTION OF PITRESSIN AND PITOCIN UPON THE CREATININE-CREATINE TRANSFORMATION AND EXCRETION IN THE BODY
(After Beard and Pizzolato (26))

Hormone	Units	No. of Rats	Exp. Days	CREATININE			CREATINE (AS CREATININE)	
				Exp., mg.	Control, mg.	Retained, mg.	Exp., mg.	Control, mg.
Pitressin	2	3	18	120	149	29	36	6
Pitressin	1 ¹	3	21	155	196	41	68	35
Pitressin	2 ¹	3	21	162	210	48	70	35
Pitressin	4 ¹	3	21	174	224	50	81	35
Pitressin	2	3	24	211	240	29	118	80
Pitressin	2	3	27	205	270	65	126	54
Pitressin	6	3	24	186	248	62	137	64
			Total			324		327
			Average			46		47
Pitocin	1	3	24	143	210	67	65	14
Pitocin	2	3	27	112	175	63	73	21
Pitocin	3	3	24	171	240	69	116	32
Pitocin	4	3	27	192	216	24	112	45
			Total			223		254
			Average			56		64
Pitocin	2	3	27	220	189	31 ²	115	36
Pitressin	1	3	18	74	72	0	28	6
Pitressin	3	3	21	110	119	9	96	14
Pitressin	4	3	21	190	154	36	164	14
Pitressin	3 ¹	3	27	197	196	0	78	28

¹ In these experiments creatinine was also injected. Since this creatinine was promptly excreted the values here also represent the retention of creatinine formed from the usual protein metabolism in the body.

² This 31 mg. of creatinine was excreted and not retained.

also of the same opinion since he stated "From the data presented we are led to believe that any effect of female sex hormones upon creatine and creatinine excretion may depend upon rapid shifts in sex steroid metabolism and accompanying rapid changes in vascular supply and in water and salt balance, rather than directly upon the amounts and kinds of hormones secreted or administered."

In 7 studies the injection of pitressin caused an average *retention* of 324 mg. of body creatinine in the rat which was accompanied by an increased creatine, as creatinine, *excretion* of 327 mg. (Beard and Pizzolato (25)). (Table 28.) In 4 other studies the injection of pitocin caused an average creatinine *retention* of 223 mg. which was accompanied by an increased creatine, as creatinine, *excretion* of 254 mg. In still other studies no creatinine was retained, but increases from 22 to 150 mg. in creatine excretion occurred. It was concluded that these posterior pituitary hormones may cause a quantitative transformation of *body* creatinine into creatine on the one hand and stimulate creatine excretion on the other. These hormones do not affect the metabolism of *administered* creatinine.

Results of studies on diabetes insipidus are conflicting. D'Antona (1) stated that the creatine metabolism is essentially normal, while Pakazody (1) observed that the creatinuria was augmented and that the creatinine coefficient was raised. Posterior pituitary treatment diminished the polyuria and creatinuria. Hanssen (1) found that posterior pituitary powder reduced the diuresis and gave an enormous extra excretion of creatinine in the urine. Iversen, *et al.* (1) and Poulsson (1) have shown that when diabetes insipidus is checked by pituitrin the creatinine clearance is altered little (Seringe (1)).

It may be concluded from the above evidence that there exists a close relationship between creatinine, water and creatine metabolism.

CHAPTER XI

CREATININE COEFFICIENT AND CREATININE CLEARANCE

Creatinine Coefficient. Prior to the studies of Folin (1, 3) and Folin and Denis (9) the blood and urine creatinine was believed to originate mainly from protein metabolism. The method of creatinine determination, before Folin (1, 3) introduced his refinements of the Jaffé reaction in 1905, was largely that of Neubauer (1) which was considered to be somewhat crude and unreliable. Using this method, however, many investigators about 1860-70 observed variations in the daily creatinine excretion in healthy men varying from 0.5 to 1.5 gm. (Voit (1); Munk (1); Neubauer (2); Hoffman (1); Hunter (1)).

Since 1905 it has been generally believed, after Folin, that the creatinine excretion is constant in a given individual if his diet is free from creatine or creatinine. Beard (1, 30) observed in 1925 that the creatinine nitrogen excretion in 400 normal medical students ingesting a normal protein diet ranged from 0.7 to 1.9 gm. per day. Later Jeltnick and Looney (1) showed that the creatinine nitrogen of whole blood of 59 hired subjects varied from 0.5 to 1.5 mg. per 100 cc. and their blood creatine varied from 1 to 1.6 mg. per 100 cc. The daily creatinine excretion varied from 0.62 to 2.29 gm. in 54 of these subjects. Cameron (1) and Beard, *et al.* (22) showed that, even in normal individuals, the re-

sponse to the ingestion of 1 gm. of creatinine varied within wide limits. Many other workers have observed these same results.

From these results it is clear that, even though the creatinine elimination may be fairly constant from day to day in a given individual ingesting a normal protein diet, this is by no means the case when many different individuals are compared. This also indicates that, contrary to the teachings of Folin and many others, the body weight cannot be a deciding factor in creatinine elimination in man.

TABLE 29

CORRELATION COEFFICIENTS OF CREATININE NITROGEN
OUTPUT WITH SEVERAL BODY MEASUREMENTS.
NORMAL DIET
(After Beard (1))

<i>Variable</i>	<i>Cases</i>	<i>r</i>	<i>P.E._r</i>
Creatinine N and Body Weight	401	0.13 ± 0.03	4.3
Creatinine N and Body Weight 2/3	401	0.13 ± 0.03	4.3
Creatinine N and Surface Area	288	0.06 ± 0.04	1.5
Creatinine N and Body Weight	122	0.09 ± 0.06	1.5
Creatinine N and Height	122	0.00 ± 0.06	0.0
Creatinine N and Body Weight (men)*	94	0.24 ± 0.07	3.4
Creatinine N and Body Weight (women)*	98	0.26 ± 0.06	4.3

* Observations taken from the literature.

The writer was probably the first to question the physiological significance of Folin's creatinine coefficient (1, 17, 30). Using 400 normal medical students, no relation was found between the creatinine excretion and their body weight ($r = 0.13 \pm 0.03$) (Table 29). Identical results were obtained with another 122 students in cooperation with Professor Alfred Chanutin of the University of Virginia, working in the Cleveland laboratory. The same was true when

100 cases of creatinine excretion and body weight were collected from the literature. On the other hand, and again contrary to the teachings of Folin, a good correlation ($r = 0.44 \pm 0.03$) was obtained in these same students when they ingested a diet rich in protein. On the normal and low protein diets there was no relation between creatinine excretion and that of total nitrogen ($r = 0.11 \pm 0.03$) on both diets. In view of these findings Beard (1) and Beard and Jacob (17) concluded that the creatinine coefficient possessed little, if any, physiological significance.

Other evidence points in this same direction. For instance, Garot (23) from Terroine's laboratory, concluded that the creatinine excretion of infants was not proportional to the muscular mass. Terroine and Garot (20) observed that the daily creatinine excretion in the rat was 60 mg. per kilo as compared to 16 mg. per kilo in the horse. It was also believed that the excretion per kilo body weight varied greatly among different warm blooded animals. It was concluded that the excretion of creatinine was in no way influenced by the muscular mass. Chanutin and Kinard (5) did not find any relation between the concentration of muscle creatine and the creatinine coefficient in the rat. The reason why a muscular 200 pound individual may excrete more creatinine than a 100 pound individual is not due to the differences in body weight, but to the fact that the larger man would evidently *eat more protein* in his larger daily food intake than the smaller man would.

The following correlation coefficients have been published by the author (1, 30) (Table 30) and compared with those of C. P. White (1) and Pucher, *et al.* (1), between the excretion of various urinary constituents (Table 31). These correlations show that protein, purine and creatinine metabolism are closely linked in the body.

TABLE 30

SHOWING CORRELATION COEFFICIENTS BETWEEN DIFFERENT FORMS OF
NITROGEN IN 400 URINES
(After Beard (30))

Variables	Low Protein Diet				Normal Diet				High Protein Diet			
	r	$P.E._r$	\pm	r	$P.E._r$	\pm	r	$P.E._r$	\pm	r	$P.E._r$	\pm
Total N, Urea N	0.91	91	0.01	0.95	0.03	0.03	0.95	0.03	0.03	0.95	0.03	0.03
Total N, Ammonia N	0.24	8	0.03	0.17	0.03	0.03	0.17	0.03	0.03	0.35	0.03	0.03
Total N, Uric Acid N	0.13	4	0.03	0.17	0.03	0.03	0.17	0.03	0.03	0.39	0.03	0.03
Total N, Creatinine N	0.10	3	0.03	0.10	0.03	0.03	0.10	0.03	0.03	0.44	0.03	0.03
Urea N, Ammonia N	0.24	6	0.04	0.20	0.03	0.03	0.20	0.03	0.03	0.31	0.03	0.03
Urea N, Uric Acid N	0.20	7	0.03	0.38	0.03	0.03	0.38	0.03	0.03	0.42	0.03	0.03
Urea N, Creatinine N	0.10	5	0.02	0.03	0.04	0.04	0.03	0.04	0.03	0.32	0.03	0.03
Uric Acid N, Creatinine N	0.22	6	0.04	0.31	0.03	0.03	0.31	0.03	0.03	0.41	0.03	0.03
Ammonia N, Creatinine N	0.13	3	0.04	0.16	0.03	0.03	0.16	0.03	0.03	0.09	0.03	0.03

TABLE 31

COMPARISON OF COEFFICIENTS OF CORRELATION BY
DIFFERENT INVESTIGATORS
(After Beard (30))

Variables	White Hospital Patients	Pucher 5 Normal Subjects	BEARD 400 NORMAL SUBJECTS 400 SAMPLES		
	50 Subjects	100 Samples	Low Protein	Normal	High Protein
Urea, Creatinine	0.71	0.36	0.10 \pm 0.02	0.03 \pm 0.04	0.32 \pm 0.03
Urea, Uric Acid	0.41	0.40	0.20 \pm 0.03	0.38 \pm 0.03	0.42 \pm 0.03
Urea, Ammonia	0.38	0.49	0.24 \pm 0.04	0.20 \pm 0.03	0.31 \pm 0.03
Uric Acid, Creatinine	0.27	0.54	0.22 \pm 0.04	0.31 \pm 0.03	0.41 \pm 0.03

Stearns, *et al.* (1) made a study of the effect of diet in malnutrition and celiac disease. Their results in relation to increase in weight and creatinine excretion were as follows (Table 32):

TABLE 32

RELATION BETWEEN INCREASE IN BODY WEIGHT
AND CREATININE EXCRETION
(After Stearns, *et al.* (1))

Child	Per Cent Increase in Creatinine	Per Cent Increase in Body Weight
J. R.	30	30
A. A.	24	12
R. S.	15	11
S. H.	25	17
E. M.	10	15
M. R.	6	17
M. B.	5	16
H. H.	42	27
A. W.	40	35
R. W.	98	35

The conclusions of the authors were as follows: "As the study stands it seems that, with similar diets, some children tend to form muscle more rapidly (as shown by the creatinine output) than they deposit fat, while for other children, the

reverse is true. With about 3 exceptions it is seen from these data that there is no relationship between creatinine output and body weight. Also the increases in body weight are more related to deposition of water or fat, rather than to muscle, as the children were fed on high carbohydrate diets (glucose and banana). The same would be true in increases in muscle weight. The spectacular gains in body weight were due primarily to sound tissue growth."

Creatinine Clearance. This subject deals primarily with a discussion of tests of kidney function which is beyond the scope of this monograph. Results of some of the recent observations, however, would seem to have a definite bearing upon this type of test and will be briefly mentioned.

Cushny's theory is that creatinine is excreted solely by glomerular filtration and that its rate of excretion depends upon its concentration in the plasma. Its excretion is also influenced by the volume of urine and other physiological factors. It has long been known that creatinine is concentrated to a greater extent than any other substance that the kidney is called upon to excrete. This fact led Rehbarg (1) to introduce the "creatinine clearance test" of renal function, which is the number of cc. of plasma cleared of creatinine per minute.

It has become customary to measure renal function in terms of volumes of blood "cleared" of some substance, *e.g.*, urea or creatinine, when passing through the kidneys. Five gm. of creatinine are ingested and simultaneous blood and urine concentrations of creatinine are measured in the test. It should be kept in mind that it is *administered creatinine* that is used in the test, and not the creatinine that arises from the amino acids during the usual course of protein metabolism.

H. W. Smith (1) has reviewed these clearance tests in his monograph of the Physiology of the Kidney. Several

observers have reported rather wide variations in the creatinine clearance tests in normal subjects. For instance Hayman, *et al.* (1) obtained values ranging from 70 to 238. Such values are to be expected in view of wide variations in creatinine retention and excretion after its ingestion by man.

Arkin, *et al.* (1) have discarded the exogenous creatinine clearance test of kidney function since in man creatinine is secreted when the blood level is artificially raised (Smith (1), Miller and Winkler (6), Shannon (1), Talbot (2)). The ideal test of kidney function could be determined by studying the excretion of inulin, or of any other substance that does not undergo any metabolic change in the body. It is now incorrect to say that creatinine and urea are waste products of metabolism. This is only partially true. Creatinine can be changed into creatine in the body. It may be oxidized and only a part of it is excreted as such in the urine. In contrast to creatine, its ingestion can also serve to increase the energy output in man. Urea can also supplement a considerable portion of the protein requirement of ruminants (*Cf.* Chapter VI). Even ammonia and uric acid can be transformed into creatine. Therefore the amounts of these constituents that are excreted into the urine probably represent an excess of each substance formed and not needed in metabolism for other functions.

Due to the variations in the creatinine content of the blood and urine after its ingestion, Cameron (1) came to the conclusion that the ingestion of creatinine was unsuitable as a test for kidney function. Ingested creatinine may be rapidly excreted or it may be retained for long periods of time in different individuals (Beard and Jacob (17)). Its ingestion also *stimulates* the excretion of both creatine and creatinine in the urine (Beard (32)). Miller and Winkler (6) have shown that the amount of creatinine secreted by the human renal tubules, estimated by the difference between the creat-

inine and inulin clearances, varies within wide limits. H. W. Smith, *et al.* (1) stated that the apparent clearance of endogenous chromogenic substances (Jaffé reaction) differs markedly when different methods of precipitation of plasma proteins are used and that the endogenous chromogen clearance is never identical with the inulin clearance.

The use of creatinine as a measure of glomerular filtration depends upon the acceptance of the theory that creatinine is excreted only by filtration through the glomerulus and undergoes no reabsorption in the tubules. It is also generally believed that a constant amount of creatinine in the blood causes a constant amount of creatinine to be excreted into the urine. On the basis of Rehburg's theory such a constant rate of creatinine excretion implies a constant rate of glomerular filtration. According to L. F. Davenport, *et al.* (1) this seems unlikely due to the physiological variations which may influence the rate of filtration through the glomerular capillaries, such as changes in blood flow, blood pressure, number of open capillaries, etc. These workers do not accept the theory of Rehburg and from their work on dogs came to the conclusion that their observations do not support the use of the creatinine determinations in blood and urine as a measure of glomerular filtration.

Abdon (1) has shown that, after the administration of either creatine or creatinine, the creatine phosphate content of the blood increases. Since administered creatinine may be transformed into creatine and stimulate the excretion of both creatine and creatinine in the urine, the writer believes that renal physiologists should, in the future, determine the "total creatinine clearance" rather than the preformed clearance, after the administration of creatinine. It should be remembered that the body metabolizes endogenous creatine and creatinine in a different manner from administered creatine and creatinine (E. Wang (1)).

CHAPTER XII

RELATION BETWEEN CREATINE- CREATININE EXCRETION WITH ENDOGENOUS AND BASAL METABOLISM

TALBOT, *et al.* (1, 2, 3) believe that there is a relationship between creatinine excretion and basal metabolism and have proposed the following creatinine standards:

Boys: Caloric output = $0.675 \times$ creatinine excretion/24 hours + 822.

Girls: Caloric output = $0.675 \times$ creatinine excretion/24 hours + 739.

In 1932 Beard (1) observed no relation between the creatinine output and surface area (and hence basal metabolism), two-thirds power of the body weight, and the height in 400 male medical students fed on a normal protein diet. It was predicted that no relation would be found between creatinine output and basal metabolism. Brøchner-Mortensen and Møller (1) reviewed the literature on creatinuria and thyrotoxicosis. No relation was observed between the excretion of creatine and creatinine in their 51 patients. Brøchner-Mortensen and Møller (2); Kepler and Boothby (6); Røddland and Wang (2); Richardson and Shorr (1); E. Wang (1); C. C. Wang (3); Rothbart (1); E. Gros (1); Pyle (1); Guhr (1); and E. Gros (1) have likewise found no relation between creatine excretion and basal metabolism.

Hess (1), Means (1), Richardson and Shorr (1), and Thorn (3) stated that thyroid treatment in myxedema increases creatinuria before the rise in the basal metabolic rate. An inverse relationship or proportionality has been reported between the creatine content of the urine and the basal metabolic rate and there is a decrease in creatinine excretion in cases of exophthalmic goiter (Eimer (3), Feldmann and Wilhelm (1, 2), Hedrich (1), Bansi (1), Sohval, *et al.* (1)). Fan (1) showed that neither the creatine nor creatinine excretion in hypothyroidism could be used as standards for the basal energy metabolism.

Endogenous Metabolism. Distinction in the past has been made between the exogenous and endogenous sources of urinary nitrogen. The exogenous metabolism was considered by Folin (1, 2, 3) to be the metabolism of all proteins ingested in excess of that required by the tissues for growth and maintenance, while the endogenous metabolism represents the breakdown of tissues and the end products are creatinine and neutral sulfur. The chief criterion of the endogenous metabolism was the constancy of the creatinine excretion which Folin believed was not influenced by the protein ingestion. While this may be true on a normal protein diet, the evidence presented in this monograph shows *conclusively* that creatinine does arise from the amino acids of the diet.

The author has never believed in any chemical and physiological distinction between these two types of metabolism. It is well known at the present time that both creatine and creatinine can be derived from proteins and amino acids no matter if these are ingested as such or if they are derived from tissue proteins. Mitchell, *et al.* (2) stated that under normal dietary conditions there may be no breakdown of tissue. Borsook and Keighly (1) have introduced the term "continuing metabolism" which is represented by the nitro-

gen metabolized on any day which is already present in the tissues, distinguishing it from the exogenous nitrogen. It is also not related to the endogenous or exogenous metabolism.

Probably the first definite evidence against any distinction between the exogenous and endogenous metabolism was published by the author (Beard (1, 17, 30)) from experimental data secured in 1925. The data are given in Tables 29 and 30, and show that there is no relation between body weight, height or surface area in the students studied or in the cases taken from published data of others. Since the heat produced in the body is proportional to the surface area (rather than the body weight) and since creatinine excretion is not related to either of these factors, it is clearly seen that the creatinine excretion cannot be used as a measure of the endogenous or basal metabolic rate, and is thus opposed to Folin's theory of the endogenous metabolism in the body (Cf. Chapter XIII on Thyroxin).

Schoenheimer, *et al.* (10) have offered definite and convincing evidence against Folin's differentiation between the exogenous and endogenous metabolism. They stated, "It is scarcely possible to reconcile our findings with any theory which requires a distinction between these two types of nitrogen. It has been shown that nitrogenous groupings of tissue proteins are constantly involved in chemical reactions; peptide linkages open, the amino acids liberated mix with others of the same species of whatever source, diet or tissue. This mixture of amino acid molecules, while in the free state, takes part in a variety of chemical reactions; some reenter directly into vacant positions left open by the rupture of peptide linkages: others transfer their nitrogen to deaminized molecules to form new amino acids. These in turn continuously enter the same chemical cycles which render the source of the nitrogen indistinguishable. Some body con-

stituents like glutamic and aspartic acids and some proteins like those of liver, serum, and other organs are more actively involved than others in this general metabolic mixing process. The excreted nitrogen may be considered as a part of the metabolic pool originating from the interaction of dietary nitrogen with the relatively large quantities of reactive tissue nitrogen."

Bloch and Schoenheimer (7) showed that both creatine and creatinine arose from the amino acids arginine and glycine in the body. In regard to the distinction between the different types of metabolism they stated, "The finding that the newly formed creatine (and creatinine) molecules acquire their parts from food as well as from tissue components is taken as further evidence against the concept of two independent (exogenous and endogenous) types of catabolism."

Mitchell (2) has recently reviewed the discussion of the significance between the endogenous metabolism of Folin and the lack of distinction between the exogenous and endogenous metabolism by Schoenheimer, *et al.* discussed above. He stated, "Concerning the reality of a constant destruction or degradation of nitrogenous tissue constituents, the endogenous catabolism of Folin, there appears to be nothing in Schoenheimer's findings inconsistent with this theory. In fact the recent studies of creatine metabolism by his group picture a reaction typical of those that must be involved in the endogenous catabolism. Tissue creatine is very constantly undergoing dehydration to creatinine (a reaction which the writer has never shown to occur in the rat or man) which is eliminated by the body as a useless metabolite (a statement to which the writer also does not agree). It is being as constantly formed, and the rate of its synthesis cannot be readily accelerated by an overabundance of its precursors in the tissues, nor by the administration of amino acids." (This last statement is certainly not accepted by the

writer and others who are convinced that creatine does take origin from the amino acids of the diet). Mitchell continues: "The summation of these constant catabolic reactions, involving the nitrogenous constituents of the tissues, may be considered to be the endogenous catabolism of Folin. The reality of these reactions is not negated by any of the facts that Schoenheimer's group has revealed."

The writer is interested in the question of endogenous and exogenous metabolism chiefly from the standpoint of creatine and creatinine metabolism. It is difficult to understand why Mitchell still refers to "these constant catabolic reactions" which usually do not occur in the body. Certainly the evidence presented in this monograph by several writers shows that creatinine excretion is only relatively constant and that its excretion does in many cases vary with the protein in the diet. Creatine and creatinine represent *uniform* rather than *constant* metabolites, and their formation and excretion are governed by the rate of protein metabolism. The writer does not believe that Mitchell has offered evidence to refute Schoenheimer's views in regard to exogenous and endogenous types of metabolism in the tissues.

CHAPTER XIII

CREATINE-CREATININE METABOLISM AND THE HORMONES

IT is generally believed that creatine and creatinine metabolism are influenced by the different hormones. It is, however, next to impossible to evaluate this relationship at the present time. Very few investigators have controlled the increases in the Jaffé reaction with alkaline picrate in the urine with the creatinine enzyme of Miller and Dubos so that it is not certain that these increases are really due to creatine and creatinine. Two examples of this may be cited here. We observed that the injection of epinephrin always gave a creatinuria which was accompanied by glycosuria. When these urines were tested with the specific creatinine enzyme of Miller and Dubos, however, it was found that the color of the Jaffé reaction persisted, showing that the Jaffé reaction after epinephrin injection was evidently due to the sugar present in the urine. It is well known that sugar reduces sodium picrate to sodium picramate which gives a red color which cannot be distinguished from that produced by creatinine in alkaline picrate unless the enzymatic method is used. This finding throws considerable doubt on the theory of Brentano (1-7) that a breakdown of muscle glycogen is the general cause of creatinuria. The other example was that an intense creatinuria occurred in the rat after insulin injection.

tions and it is impossible to believe that there was any breakdown of glycogen in this case.

Practically no investigator has tested his endocrine preparations to see if they themselves would give the Jaffé reaction with alkaline picrate. We found that theelin gave a slight Jaffé reaction and Andostine A (a total testicular extract of the Ciba Products Company) gave an intense Jaffé reaction with alkaline picrate. Also no studies except our own have been made to observe the effect of trauma, of the anesthetic used in operative procedures, or the water content of the preparations used, upon the degree of creatinuria observed in endocrine studies. We have shown that any one of these factors will temporarily increase the creatinuria in our studies. It is also very difficult to determine whether the effect of a given hormone on the creatine-creatinine metabolism is direct or indirect through the action of some other hormone.

In spite of these difficulties, however, the subject of the relation of the hormones to creatine and creatinine metabolism is an important one and will be discussed here. It will be best to simply list the recent results published in this field, as follows:

1. *Creatine excretion increases in the urine of normal animals or man after the administration of the following hormones:*

Thyroxin: Terroine and Bonnett (7); Mori (1); Pugsley, *et al.* (1); Bodansky and Duff (3); Brentano (3); Bühler (1); Carson (1); Deuel, *et al.* (8); Eimer (1); Hirst and Imrie (4); Krause and Cramer (3); Mark (1); Rathery, *et al.* (1); Sure, *et al.* (1); Fan (1); Wilkins, *et al.* (2).*

* At the same time, after the administration of thyroxin, the creatinine excretion is usually decreased. An increased creatinuria accompanied by decreased creatinine excretion also occurs in conditions of thyreotoxi-

Thyreotrophic Hormone: Schrire and Sharpley-Shaffer (1); Pugsley, *et al.* (1); Gaebler and Bartlett (3); Luhrs (1); Bühler (1); Wilkins, *et al.* (4).

Anterior Pituitary Growth Hormone: Nitzescu and Gontzea (1).

Lactogenic Anterior Pituitary Hormone: Luhrs (1).

Follicular Hormone: Kun and Peczenik (1).

Epinephrin: Buchy (1); Medvedeva (1).

Suprarenal Cortical Hormone: Terroine and Babad (17); Babad (18); Yamase (1).

2. *Creatine excretion is not changed, is prevented or diminished*:

Anterior Pituitary Growth Hormone: Schrire and Sharpley-Shaffer (1); Gaebler and Bartlett (3).

Lactogenic Anterior Pituitary Hormone: Luhrs (1).

Posterior Pituitary Hormones: Bühler (1).

Testosterone Propionate: Coffman and Koch (1); Bühler (2); Kenyon, *et al.* (2, 3); Jailer (1); Williamson and Gulik (1); Buadze (7); Duckworth (1).

Andosterone: Cheetam and Zwarenstein (3).

Estradiol Benzoate: Jailer (1).

Parathyroid and Adrenal Hormones: Berman (1).

Suprarenal Cortex Hormones: Terroine and Babad (17); Babad (18).

Thyroidectomy: Allison and Leonard (1).

cosis and after administration of thyroid gland substance or dessicated thyroid. In these cases there is a reduction in the creatine content of the heart (Bodansky (4); Bodansky and Duff (5); Bodansky (6); Bodansky and Pilcher (7); Bodansky, *et al.* (4, 8); Cowan (1); in the creatine phosphate content of the heart (Bodansky (4); Bodansky, *et al.* (8); Buell, *et al.* (1); Fieschi and Gavazzeni (1); Mattonet (1); and in the creatine content of striated muscle (Abeilin and Spichtin (1); Bodansky (4); Bodansky, *et al.* (3, 5, 9); Osada (1); and Shaffer (1)). From these results it is clear that there is no relation between creatinine excretion and the endogenous metabolism which was first advanced many years ago by Folin. The creatinuria of thyreotoxicosis is often reduced by the administration of iodine.

3. *Creatinuria occurs in castrates*: Read (1); McNeal (1); Remen (1); Bühler (1); Macciotta and Studi-Sassar (1); Querol and Reuter (1); Seghini (1).

4. *Creatinuria does not occur in castrates*: Tsun-Chee Shen (1); Kochakian and Murlin (3); Sandberg, *et al.* (1).

5. *Creatine excretion is increased in castrates*:

Male Sex Hormone: Bühler (1).

Testes Extract: Kyogoku (1).

Follicular Hormone: Kun and Peczenik (1); Seghini (1).

Suprarenal Cortical Hormone: Terroine and Babad (17).

Estrogens: Allison and Leonard (1).

6. *Creatine excretion is decreased in castrates*: Bühler (3); Schittenhelm and Bühler (4); Usui, *et al.* (1).

7. *Creatinuria occurs in Addison's Disease*: Schittenhelm and Bühler (5); Tiemann (1). (Scopinaro (1) also showed creatinine retention.)

8. *Removal of the suprarenals results in a loss of creatine phosphate from the muscles*: Cope, *et al.* (1); Kuschinsky and Nachmansohn (1); Lang (1); Lundsgaard and Wilson (2); Maranon, *et al.* (1); Moschini (1); Nachmansohn and Kuschinsky (2); Ochoa (1); Ochoa and Grande (2).

9. *Androgens cause a decrease in hypogonad creatinuria*: Bühler (3); Kun and Peczenik (1); Paschkis and Schwoner (1); Kenyon, *et al.* (2).

10. *Creatinine excretion is increased in normal animals or man*:

Growth Hormone of the Anterior Pituitary: Schrire and Zwarenstein (4).

Gonadotrophic Hormone: Schrire and Sharpley-Shaffer (1); Luhrs (1).

Lactogenic Anterior Pituitary Hormone: Luhrs (1).

Anterior Pituitary Extracts: Bühler (1).

Lipoid Extract of the Testes: Cheetam and Zwarenstein (3).

Testosterone Propionate: Cheetam and Zwarenstein (3).

Andosterone: Cheetam and Zwarenstein (3).

Epinephrin: Medvedva (1).

Thyroxin: Mori (1).

Thyroidectomy: Coffman and Koch (1).

11. *Creatinine excretion is not changed or is diminished*:

Thyreotrophic Hormone: Luhrs (1).

Anterior Pituitary Growth Hormone: Schrire and Sharpley-Shaffer (1).

Lactogenic Anterior Pituitary Hormone: Luhrs (1).

Saline-Suspension of Testes: Cheetam and Zwarenstein (3); Kyogoku (1).

Male Sex Hormone: Bühler (1).

Testosterone Propionate: Jailer (1); Williamson and Gulik (1); Coffman and Koch (1).

Estrogens: Sharpley-Shaffer and Schrire (2); O. W. Smith (1).

Thyroxin: Coffman and Koch (1).

Thyroid: Fan (1).

Suprarenal Cortical Hormone: Babad (19); Yamase (1).

Thyroidectomized Rabbits: Medvedva (1).

Thyreotrophic Hormone or Thyroid Administered to Normal and Hypothyroid Children: Wilkins and Fleischmann (4).

Nathanson, *et al.* (1) showed that in growing children, both boys and girls, there is a close correlation between creatinine excretion and of 17-ketosteroids. They believe that the androgens have an influence on muscle mass of the individual so that the increase in androgen formation would result in an increase in muscle mass and, hence, of creatinine excretion.

In all of our studies in this field we have injected the hormone preparations once. Changes in creatine-creatinine metabolism were observed soon afterwards. The action of the hormones on creatine-creatinine metabolism may be di-

rect, or indirect through the action of the injected hormone upon other endocrine glands. Our results using rats may be summarized as follows:

1. Normal rat + sex hormones (testosterone propionate, theelin, progestin, also Antuitrin T and G) → Creatinuria (probably due to hydration of creatinine to creatine, since in all cases there was a retention of water or salt) (Pizzolato and Beard (18); Wilkins, *et al.* (5)).

2. Normal rat + sex hormones + creatinine → Creatinuria (due to the hydration of creatinine to creatine and also to the stimulating effect of creatinine upon creatine and creatinine excretion (Beard and Jacob (23))).

3. Normal rat + sex hormones + creatinine → Creatinuria (the stimulating effect of creatinine upon creatine excretion is increased in the presence of either of the male or female sex hormones (Beard and Jacob (23))).

4. Normal rat + male or female sex hormones → Creatinuria (but no increased creatinine excretion (Beard and Jacob (23))).

5. Normal rat + creatine → Creatinine retention (Beard and Jacob (23)).

6. Normal rat + creatine + sex hormones → No increase in creatinine excretion (Beard and Jacob (23)).

7. Normal rat + creatinine + sex hormones (oily solution mixed in syringe with creatinine solution in water) → No creatinuria (Beard and Jacob (23)).

8. Normal rat + creatinine + sex hormones (injected separately) → Creatinuria (there was a summation of creatine excretion in these cases) (Beard and Jacob (24)).

9. Recent castrates → Creatinuria (some of which is due to the trauma and anesthetic used in the operation) (Pizzolato and Beard (18); Koven and Beard (29)).

10. Women after hysterio-öophrectomy → Extensive creatinuria (Pizzolato and Beard (18)), some of which could

have been due to the trauma and anesthetic of the operation (Koven and Beard (29)). Creatinuria disappears about 2 weeks postoperatively.

11. Recent castrates + sex hormones (theelin, testosterone propionate, progestin, also Antuitrin T and G) → Creatinuria, more intense in the castrates as compared to the same animal before castration (Pizzolato and Beard (18); Koven and Beard (19)).

12. Three and 6 month castrates + sex hormones + creatinine → Creatinuria (due to the effect of injected saline alone) (Beard, *et al.* (22)).

13. Normal, recent and old castrates + saline → Creatinuria due to the hydration of creatinine to creatine (Beard, *et al.* (22); Beard and Jacob (24)).

14. Adrenalectomy → Creatinine retention for 3 days followed by creatinuria (Koven and Beard (29)).

15. Normal rat + adrenal cortical hormone (eschatin or percorten) → Creatinuria (Koven, Pizzolato and Beard (25)), possibly due to retention of salt and water (Krohn and Zuckerman (2); Thorn and Engel (1); Thorn and Harrop (2)). (Color of the Jaffé reaction was destroyed by the Miller and Dubos creatinine enzyme.)

16. Normal rat + epinephrin → Glycosuria (color of the Jaffé reaction was *not* destroyed by the M and D specific creatinine enzyme (Koven, Pizzolato and Beard (25))).

17. Normal rat + creatinine → Stimulation of both creatine and creatinine excretion (Beard and Pizzolato (26)).

18. Normal rat + pitressin → Stimulation of creatine excretion and retention of creatinine (Beard and Pizzolato (26)).

19. Normal rat + creatinine + pitressin → Creatinuria, one-third greater than that obtained from creatinine alone (Beard and Pizzolato (26)).

20. Normal rat + pitressin → An average retention of

324 mg. of body creatinine, was accompanied by an increased excretion of creatine, as creatinine, of 254 mg. (Beard and Pizzolato (26)).

21. Normal rat + creatinine + pitressin → *Injected creatinine*, was excreted as such with a stimulation of both creatine and creatinine excretion. Later *body creatinine was retained and was quantitatively excreted as creatine* (Beard and Pizzolato (26)).

22. Normal rat + creatinine + pitressin → Body creatinine retention roughly proportional to creatinuria (Beard and Pizzolato (26)).

23. Normal rat + pitocin → Average body creatinine retention of 223 mg., accompanied by an increase in creatine excretion of 254 mg. (Beard and Pizzolato (26)).

24. Normal rat + pitressin or pitocin → No body creatinine retention so the creatinuria was due to the *stimulating effect* of these hormones on creatine excretion (Beard and Pizzolato (26)). (This is an illustration of *variations* in creatine-creatinine excretion that may be observed under identical experimental conditions. It is of much interest in this connection that Ohashi (1) observed that pitressin reduces urea excretion.)

25. Thyroidectomy → Creatinuria (probably due to the trauma and anesthesia used in the operation) (Koven and Beard (29)).

26. Normal or thyroidectomized rat + desiccated thyroid → Increase in both creatine and creatinine excretion (Beard and Pizzolato (26)). (The fall in tissue creatine during hyperthyroidism is prevented by feeding a high protein diet (Wyss (1)).)

27. Normal rat + parathyroid extract → Creatinuria (evidently due to water content of preparation (Beard and Pizzolato (26)).

28. Normal rat + insulin (iletin) or zinc insulin (crys-

tals) → The creatinuria and creatininuria were greater in the case of zinc insulin than was the case after insulin (Beard and Pizzolato (26)). The action here was probably due to the action of these insulins on protein and carbohydrate metabolism.

29. The addition of thyroxin, pitressin, or pitocin, to creatinine solutions in the presence of different tissues, did not have any effect on the oxidation and hydration of creatinine (Beard (37)).

30. Normal rat + sex hormones (theelin, testosterone propionate (also A. P. extract (growth, thyrotrophic and sex hormones)) → Increase in muscle creatine (Pizzolato and Beard (18)). (Kato (1) observed increases in creatine content of the rabbit uterus, and Yamase (1) in the walls of the vagina, during pregnancy.)

31. Castrate male and female rats → No change in muscle creatine (Pizzolato and Beard (18); Koven and Beard (19)).

32. Normal rat + progestin → No change in muscle creatine (Koven and Beard (19)).

33. Normal rat + epinephrin or eschatin → No change in muscle creatine (Koven, Pizzolato and Beard (25)).

34. Normal rat + pitressin or pitocin → Increase in muscle creatine (Beard and Pizzolato (26)).

35. Normal rat + insulin, zinc insulin, desiccated thyroid, or parathyroid extract → No change in muscle creatine (Beard and Pizzolato (26)).

Two suggestions may be offered here to explain the above results: (a) The creatinuria as stated above may be due to water and salt retention after the injection of some of the above hormones (Krohn and Zuckerman (1); Thorn, *et al.* (1, 2); O. W. Smith (1); and Zuckerman (2)), or (b) to the increase in blood amino acids after the injection of the growth and metabolic factors of the anterior pituitary, pitres-

sin, antuitrin-S, testosterone propionate, and thyroxin (Farr and Alpert (1)).

The results of several other studies should be mentioned here. Medvedva (1) showed that adrenalectomy does not affect the water or protein content of the skeletal muscles, brain, or liver of the rat. Administration of cortin raised the water content of the muscles. Male rabbits after administration of testes powder and also after castration showed an increase in residual ammonia, nitrogen, urea, creatine and amino nitrogen in the skeletal muscles (Osada (2)). The creatinine content of skeletal muscles of female rabbits remained unchanged after oral administration of interstitial powder, of corpus luteum powder or after oophrectomy. Creatine, amino acid nitrogen, ammonia, urea, and lactic acid increased after administration of interstitial powder, but creatine decreased after corpus luteum powder and after oophrectomy (Osada (3)). A diminution of the creatine-creatinine content of the liver, skeletal muscles and heart in experimental hyperthyroidism was prevented if the animals were fed on a diet rich in protein and fat (Wyss (1)).

Mirsky (1) discussed the rôle of insulin in protein metabolism. It inhibits deamination of amino acids in the liver and increases the utilization of the amino acids by the muscles. During the insulin treatment of 3 patients with myasthenia gravis one case showed an increase of 600 per cent in general muscle power. This effect was lost when insulin was omitted. Robinson (1) stated that a case of myasthenia gravis which showed no response to prostigmin did so when insulin was given in addition.

Koch (4) reviewed the literature on the effect of the male sex hormone and Coffman and Koch (1) the effect of testosterone propionate on creatine-creatinine metabolism. Kenyon, *et al.* (2, 3) showed a definite increase in weight in their eunochoids during treatment with testosterone propionate,

some of which was stated to be due to a storage of salt and water (Krohn and Zuckerman (1); Thorn, *et al.* (1, 2); Zuckerman (2)). According to these workers the increased retention of creatine in their patients, which paralleled the gain in weight, indicates an increased muscle production under the influence of the male sex hormone. It is also of much interest in this connection that Papanicolaou and Falk (1) showed that the temporal muscles of male guinea pigs are larger than those of female pigs and that a muscular hypertrophy was produced in male and female castrates by the administration of testosterone propionate.

Paschkis and Schwoner (1) showed a nonspecific action of the male hormone on creatine metabolism. They, however, used the commercial preparation, Andostine, which the writer has shown gives an intense Jaffé reaction itself with alkaline picrate.

Sherman (1) showed that in castrated animals there was a reduction of about 40 per cent in creatine phosphate and glycogen content of the heart muscle.

Brown and Imrie (1) showed that the creatine varied with the creatine phosphate and total inorganic phosphate content of cat muscle. Imrie and Jenkinson (2) and Brown and Imrie (3) showed that removal of the thyroid and parathyroids caused a lowering of 50 per cent in the creatine phosphate content of cat muscle and that the normal rate of recovery (about 1 hour) was much delayed. Phosphorus retention which occurs after creatine administration was more pronounced after injection of parathormone. It would seem, therefore, that the parathyroids exert an influence over creatine phosphate metabolism.

Pyle (1) studied the relation between Hb, B.M.R. creatine and creatinine and Mg in 5 pregnancies. His results (Table 33) were as follows:

TABLE 33

Hb, Mg, B.M.R., CREATINE AND CREATININE
IN PREGNANCY
(After Pyle (1))

<i>Variables</i>	<i>r</i>	<i>P.E._r</i>
Creatine-oxygen consumption	-0.19	0.14
Creatinine-oxygen consumption	+0.34	0.12
Creatine-urinary Mg	+0.48	0.09
Creatinine-urinary Mg	-0.22	0.13
Creatine and Creatinine	-0.56	0.09

The 24 hour excretion of preformed creatinine has been measured by O. W. Smith (1) in 6 menstruating women (140 determinations during 14 cycles); in 5 women with gynecological disorders to 4 of whom estrogenic, progestational or gonadotrophic hormones were administered (56 determinations); in 1 woman before and during the cycle of conception and during the first two months of pregnancy complicated by threatened abortion (27 determinations); in 10 women during the course of normal pregnancy (80 determinations); in 4 women during labor (19 determinations); and in 9 women who developed preeclampsia, to 6 of whom large amounts of estrogen, progestin, testosterone or pituitary gonadotrophic hormone were administered (81 determinations). Urinary creatine was measured in 4 menstruating women (113 determinations during 11 cycles). Urinary estrogens were also measured in all specimens, the gonadotrophic potency of the urine in many and urinary pregnandiol in those from pregnant women.

In menstruating women there was evidence that, although ovarian secretion did not directly affect creatine and creatinine excretion, a definite change in creatine and creatinine metabolism was associated with the menstrual phenomenon itself.

Neither gynecological disorders nor the administration of hormones to non-pregnant women in amounts sufficient to cause physiological changes and markedly to affect hormone excretion, appeared to influence the creatinine output.

The only consistent or significant change in urinary creatinine in normal pregnancy was found during the course of labor, when a striking drop in the rate of its excretion was encountered.

Creatinine excretion was not demonstrably affected by the development of preeclampsia despite the accompanying marked changes in hormone metabolism, or by the large amounts of sex hormones administered to patients with the disease.

Smith stated: "From the data presented we are led to believe that any effect of female sex hormones upon creatine and creatinine excretion may depend upon the rapid shifts in sex steroid metabolism and accompanying rapid changes in vascular supply and in water and salt balance, rather than directly upon the amounts and kinds of hormones secreted or administered." (Cf. theories of the author in Chapter X.)

Five dwarfed, sexually undeveloped boys were treated with methyl testosterone by mouth in doses of 25 mg. daily, by Wilkins, *et al.* (5). Two dwarfed, sexually undeveloped girls were given 1 mg. stilbestrol and 25 mg. methyl testosterone by mouth daily. The administration of methyl testosterone caused retention of nitrogen which occurred within the first one to three days. On an average of ten days after the beginning of treatment, an increase of the output of creatine occurred. This creatinuria continued to increase and was at a high level after two to five months of treatment. On discontinuing methyl testosterone a nitrogen equilibrium or deficit resulted within a few days, while the creatinuria decreased gradually over a period of 16 to 26 days. In one

typogonad male treated with methyl testosterone by mouth Tager and Shelton (1) observed a rise in creatine excretion from 225 mg. daily before treatment to 541 mg. and 744 mg. during treatment.

CHAPTER XIV

RELATION OF CREATINE TO POTASSIUM, PHOSPHATE AND CARBOHYDRATE METABOLISM. CHEMISTRY OF MUSCULAR CONTRACTION. PHOSPHATE BOND ENERGY. PHOSPHORYLATION AND RESPIRATION

Creatine and Carbohydrate Metabolism. It has long been known that there is an important relation between creatine and carbohydrate metabolism. In diabetes mellitus, phloridzin diabetes and pancreatic diabetes, creatinuria occurs. Complete or carbohydrate starvation will likewise result in creatinuria which can be abolished by the feeding of sugar or protein. Increased excretion of creatine also occurs in cases of glycosuria (Cathcart and Taylor (1); Lieben and Laszlo (1); McAdam (1); Underhill and Baumann (1); and Krause and Cramer (1)). Creatine increases the blood sugar (Hill and Mattison (1); Klopwitz (1, 2); and Peabody and Hill (2)). Insulin lowers the blood creatine (Klopwitz (3, 4); Rigo and Frey (1)), and increases the creatine excretion in rats (Beard and Pizzolato (22)). Creatine greatly increases the glycogen content of the liver of the rat and this increase is still greater if cholic acid and creatine are administered together (Sugiyama (1)).

On the other hand the administration of creatine may increase the synthesis of glycogen (Reuter and Schlessmann (1); Lanari (1)). The German workers showed that after ingestion of 3 gm. of creatine by normal persons creatinuria occurred. This was diminished by the simultaneous administration of glucose and still more by the injection of insulin. Glucose and insulin did not affect the spontaneous creatinuria. Intravenous injection of 2 gm. of creatine caused a fall in blood sugar in diabetic patients comparable to that produced by 10 units of insulin. A combination of the two caused no greater effect. The fall in blood sugar in the diabetic patient after the ingestion of creatine is accompanied by an increased excretion of acetone bodies, presumably as the result of increased synthesis and utilization of fatty acids from glucose. Creatinuria is considered to be a sign of loss of glycogen and deficient synthesis in the muscles. A compound of creatine and glycogen was suggested (Myers and Fine (2)).

Brentano (1-7) for several years has advanced the theory that creatinuria is due to a breakdown of muscle glycogen in the body. Lohmann (1) followed up some of these observations of Brentano. He produced creatinuria in rabbits by means of urethane, glycine, hunger, thyroxin, etc. There was a diminished utilization of sugar. Lohmann also regards creatinuria to be due to a destruction of glycogen and diminished utilization of sugar. Schauf (1) likewise observed in rabbits suffering from muscular dystrophy that the injection of 1 cc. of epinephrin with administration of glycine causes a decrease in muscle glycogen. This investigator does not believe that glycine is transformed into creatine. Jahn (1, 2) observed that the ingestion of 2 or 3 gm. of creatine caused little change in the respiratory quotient, but caused a lowering of the basal metabolic rate. If a small amount of glycogen is present in the liver, creatine causes ketonemia and a

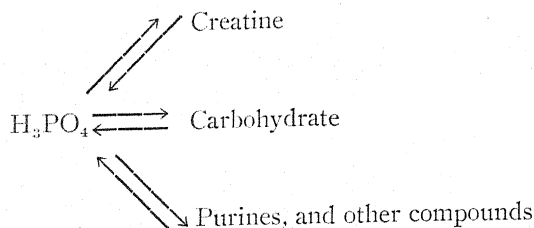
marked fall in blood sugar. Creatine reinforces the action of insulin in assisting in the formation of muscle glycogen. Brentano (7) likewise observed that during creatinuria in the rabbit there was a failure to form as much glycogen from glucose as normal. Mazzoleni (1) also observed that during creatinuria exogenous sugar was not eliminated, synthesized to glycogen, oxidized, or esterified with P_2O_5 . Murakami (1) reported that creatinine did not influence the blood sugar level in the rabbit. But if creatinine and glucose were administered together, a fall in blood sugar resulted. Injection of creatinine did not promote the formation of glycogen from glucose. Kuplowitz (1) observed a fall in blood creatine after insulin injections. Del Guerra (1, 2) showed that the increase in creatine compounds during creatinuria is related to methyl and acetyl groups which combine with creatine under special conditions.

The above evidence would seem to indicate that physiological creatinuria would be due to a disturbed utilization of carbohydrate. The author, however, does not agree with this view. In the first place simply because creatinuria and a disturbed utilization of carbohydrate may occur together does not indicate that the latter is the cause of the former. They may both be due to another factor. In the second place the theory does not account for all types of creatinuria. In the third place creatinuria occurs after sugar feeding (Haldi and Bachmann (1); Hobson (1)), and in other conditions where carbohydrate utilization is not impaired. Pollack (1) showed an increase in creatine phosphate in the dog's heart after adding glucose to the perfusion fluid and Hertz (1) showed that in glycogen storage disease, the chief abnormality was a high excretion of creatine and creatinine. In the fourth place it is very difficult to understand why the administration of glycine should cause a destruction of glycogen (Lohmann (1)) when it is well known that it pro-

motes the formation of sugar instead. In the fifth place Schauf (1) does not believe that glycine forms creatine when there is irrefutable evidence that it does so, and it is again difficult to understand why glycine in Schauf's study caused a decrease in glycogen. The breakdown here of glycogen was evidently due to epinephrin and not to glycine. In the sixth place the administration of epinephrin cannot be used in studies of this nature since this hormone itself gives the Jaffé reaction and this color is not destroyed by the specific creatinine enzyme of Miller and Dubos (*Cf.* Chapter XIII). In the seventh place creatinuria may occur in so many different conditions that E. Wang (1) stated that it was an entirely unspecific phenomenon. It is, therefore, evident that a disturbed utilization of sugar or a breakdown of muscle glycogen cannot be the chief cause of creatinuria.

In order to better understand the cause of physiological creatinuria it will be necessary to discuss the relations between creatine and carbohydrate metabolism with that of phosphorus. The evidence presented in this monograph shows that, contrary to previously accepted views, creatine is not transformed into creatinine, but, on the other hand, creatinine is transformed into creatine.* Creatinine is not phosphorylated and the excess not needed to form creatine is excreted. Creatine is phosphorylated and stored as creatine phosphate. These are steady state reactions. The amount of creatine phosphate formed then depends upon the *available phosphate* in the tissues and this brings carbohydrate metabolism into the picture.

* The author is well aware that many students of creatine and creatinine metabolism will not agree with this statement. We have never denied the possibility that creatine was changed into creatinine in the body. On the other hand we have stated repeatedly that in all our studies over a number of years *we have yet to observe this transformation.*

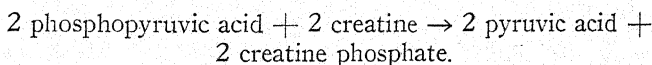


One should, therefore, forget about free creatine in the future and think only of creatine phosphate. Hence it is clearly seen that any condition, or set of conditions, that alter the general metabolic, and especially phosphate and pH equilibrium, will at the same time alter creatine phosphate equilibrium. This same view has been expressed by E. Wang (1). The level of muscle creatine would be affected by phosphoproteins (in fact creatine phosphate may be combined with the protein myosin of the muscles), sodium, potassium or calcium phosphates, glycogen, various drugs, hormones, vitamins, amino acids, purines, water, saline, changes in external temperature, etc. The degree of creatinuria would also be affected by infancy, pregnancy, starvation, removal of the gonads in animals and man, in diseases of the liver, in carbohydrate deprivation and in carbohydrate feeding. In addition to all of these effects the creatinuria which usually occurs in diseases of the muscles would be due either to a non-utilization of creatine, an increased liberation of creatine from disintegrating muscle tissue, or to an increased water content of the diseased muscles. Possibly all three factors are operating. Also in these myopathies the various phosphate fractions are lowered with the creatine content. It is also reasonable to expect that the level of muscle creatine would affect the excretion of phosphorus in the urine and feces. In fact Clarkson (1) has shown that administration of creatine by mouth causes a lowering of the phosphate excretion.

Sachs and Altshuler (3) injected radioactive phosphorus into cats. It was found that the P^{32} content of the intracellular inorganic phosphate of the heart was very much greater than the corresponding value in striated muscle. The rates of new formation of creatine phosphate and adenosine triphosphate were found to be many times as great in heart as in striated muscle. These results will be of value in elucidating the mechanism by which phosphate groups enter the muscle fiber and in studying the interrelationship between the acid soluble phosphorus compounds in the resting metabolism of striated muscle.

Brown and Imrie (1) showed that a high content of creatine in muscle was accompanied by a high concentration of creatine phosphate and total acid soluble phosphorus. Imrie and Jenkinson (2) stated that the phosphorus retention which follows creatine administration is rendered more pronounced by parathormone. According to Brown and Imrie (3) stimulation of normal cat muscle causes a loss of 50 per cent of its creatine phosphate content and the rate of recovery is much slower. Orthophosphates show reciprocal changes. Parathormone raises the creatine phosphate content to normal in these operated animals. This shows that the parathyroids are related to creatine phosphate metabolism. Del Guerra (2) stated that administration of exogenous phosphorus causes an increase in muscle creatine and inhibits the hydrolysis of creatine phosphate in the muscles.

In the hydrolysis of glycogen, hexose phosphates and phosphopyruvic acid are formed and the latter compound can yield its phosphorus in the Parnas reaction to creatine to form creatine phosphate, as follows:



The phosphopyruvic acid serves as a phosphate donator and this results in creatine retention in the muscle in the form of creatine phosphate. Instead of being the cause of creatinuria as suggested by Brentano, and others, the hydrolysis of glycogen should serve to supply phosphate donators to form creatine and thus to prevent creatinuria.

The metabolism of phosphate, therefore, should occupy the central position in any discussion of creatinuria. When this view is taken it is seen that a disturbed utilization of carbohydrate could be only one of the causes of creatinuria or associated with it. In carbohydrate deprivation there would also be a lack of phosphate donators to hold creatine in the muscles which would then result in creatinuria. The theory of the author, as discussed in Chapter VIII is that creatinuria will occur when there is, for any reason, a lack of phosphate donors in the tissues to form creatine phosphate from creatine and phosphate.

Long before the discovery of creatine phosphate by Fiske and Subbarow (1) and the Eggletons (1) evidence had been published showing a possible relation between creatine and other constituents of the muscle tissue. Urano (1) in 1906 pointed out that in *in vitro* studies creatine and phosphorus diffused from muscle tissue at about the same rate. In 1913 Professor Myers, while working upon the creatine content of rabbit muscle and its relation to urinary creatinine, was of the opinion that there was a close relation between creatine, phosphorus and potassium with glycogen in muscular activity (Myers and Fine (2)). The phosphorus analyses were unreliable, but accurate figures for potassium were obtained. The later discovery by Fiske and Subbarow (1) of the acid nature of creatine phosphate disclosed an important relationship between this substance and the acid-base balance of muscle tissue. Creatine phosphate is a stronger acid than inorganic phosphate or hexose phosphate, and its

hydrolysis would result in liberation of a base which then becomes available to neutralize the lactic acid and other organic acids formed during muscular contraction and recovery (Fiske and Subbarow (1), Sachs (1)). Probably the chief substance in muscle cells which is capable of furnishing all of the base binding capacity of creatine phosphate is potassium. Myers and Mangun (7, 8, 10) have suggested, therefore, that it is more logical to believe that creatine phosphate occurs in muscle tissue as the dipotassium salt. They have offered the data in Table 34 to show the correlation of creatine, phosphorus, and potassium in skeletal muscle.

TABLE 34

CORRELATION OF CREATINE, POTASSIUM AND
PHOSPHATE IN SKELETAL MUSCLE
(After Myers and Mangun (7))

Animal	No. of In- divid- uals	Creat- inine mg./100 gm.	P mg./100 gm.	K gm.	Creatine mM/kg.	P mM/kg.	K mM/kg.	Per Cent K* (theo- retical)
Guinea pigs	6	441	223	352	33.6	72.0	90.2	134
Humans, nor- mal cases	11	443	201	328	33.7	64.7	84.0	125
Dogs	3	451	229	356	34.4	73.9	91.1	132
Rats	3	459	246	365	34.9	79.3	93.5	134
Rabbits	3	499	244	368	38.1	78.7	94.3	124

* Theoretical, calculated on the basis of K required to account for all of the creatine in terms of the dipotassium salt of creatine phosphate.

Their data indicate that in the hydrolysis of creatine phosphate, 2 moles of potassium are formed for each mole of creatine and phosphorus. Mangun and Myers (4) showed in other studies conducted upon the creatine, phosphorus and potassium contents of human hearts in myocardial insufficiency that there was slightly more than 1 mole of phosphorus and slightly more than 1 mole of potassium lost from the heart for each mole of creatine lost. A relationship was also shown between creatine and the total acid soluble phos-

phorus in different types of muscle and brain of a variety of species of mammals (Myers and Mangun (7)).

These findings of Myers and Mangun are in accord with the known functions of potassium in metabolism. This aspect of the question has been reviewed by Fenn (2). Many investigators have shown that muscular activity leads to a loss of potassium in exchange for sodium, whose concentration in the blood increases under these conditions. No doubt the progressive loss of potassium is what causes the intensity of muscular contraction to decrease. These facts indicate that potassium is in some way connected with the contraction and recovery processes in muscle. There is also a loss of creatine phosphate during contraction, and the loss of potassium could easily accompany that of creatine phosphate. Potassium, as well as glycine (which increases muscle creatine), has been shown to be of benefit in some cases of myasthenia gravis. Potassium therefore probably plays an important rôle at the neuromuscular junction or synapse. A liberation of acetyl choline after potassium injections occurs.

Several investigators have observed an increase in response, a stimulation or an increase in excitation of skeletal muscle after the administration of small amounts of potassium. When potassium is applied in larger concentration to the muscle fiber itself it causes the familiar potassium contracture. During this contracture there is a breakdown of creatine phosphate accompanied by lactic acid formation and increased oxygen consumption. Administration of potassium is also known to increase the heart rate. In the disease known as familial periodic paralysis administration of potassium causes a relief of these symptoms. An increase in the ratio of potassium to calcium increases the tone of smooth muscle. It is therefore clear that potassium plays an important rôle in muscle physiology.

Creatine phosphate was isolated from muscle tissue by

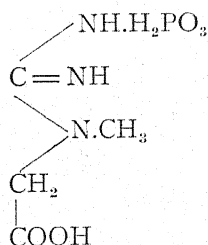
Fiske and Subbarow (1) and the Eggletons (1) and its distribution in tissues is given in Table 35 (after Lipmann (1)).

TABLE 35

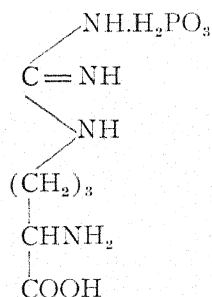
CREATINE PHOSPHATE IN TISSUES
(After Lipmann (1))

Tissue	MG./% P		Reference
	Creatine P	Inorganic P	
Muscle, frog	54	22	Gerard and Tupikova (2)
Muscle, cat	60	26	Fiske and Subbarow (1)
Muscle, amphioxus	37	72	Meyerhof (3)
Brain, dog	12	—	Gerard and Tupikova (2)
Nerve, frog	9	7	Gerard and Tupikova (2)
Heart, normal rat	4-7	—	Bodansky (4)
Heart, hyperthyroid rat	1-3	—	Bodansky (4)
Stomach, rabbit	2-5	25-32	Eggleton and Eggleton (2)
Testicle	0.6-2.6	8-12	Eggleton and Eggleton (2)
Uterus, rabbit	1.4	11.6	Eggleton and Eggleton (2)
C 180 tumor, mouse	1.5-2.7	—	Franks (1)
L.R. 10 tumor, rat	2.5	25	Boyland (1)
Jensen sarcoma	1.2	22	Boyland (1)

The Chemistry of Muscular Contraction. Before discussing this subject the structure of various compounds that play a part in muscular contraction will be given. Meyerhof and Lohmann (2) discovered arginine phosphate in invertebrate muscle. The structures of creatine and arginine phosphates are as follows:



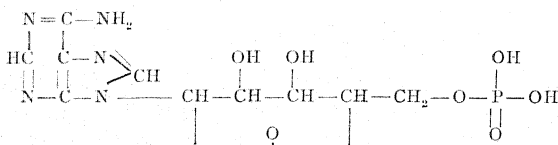
Creatine Phosphate



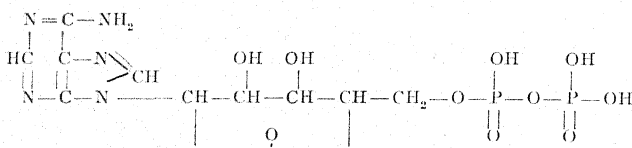
Arginine Phosphate

Muscle also contains the nucleotide, adenylic acid, which consists of the purine adenine, the pentose, ribose, and phos-

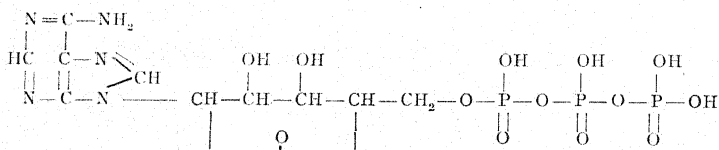
phoric acid. On hydrolysis of the nucleotide the phosphoric acid is split off leaving the nucleoside, adenosine. Adenosine diphosphate and triphosphate have also been discovered in muscle tissue. The structures of these three compounds are as follows:



Muscle adenylic acid (adenosine-5-monophosphoric acid)

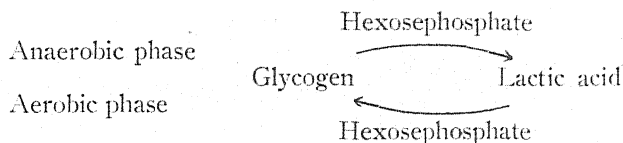


Adenosine diphosphoric acid



Adenosine triphosphoric acid

The response of an isolated muscle to stimulation is not accompanied by increased consumption of oxygen. After the contraction and relaxation are over, however, increased oxygen consumption occurs. There are then two phases of the contraction cycle, the anaerobic (without oxygen or anoxidative) and the aerobic (oxidative) or recovery phase. During the anaerobic phase lactic acid is produced from the hydrolysis of glycogen and the carbohydrate cycle enters the picture. In the aerobic or recovery phase, oxygen is consumed and a small amount of the lactic acid is oxidized, while the greater part of it is reconverted to glycogen.



Until the discovery of creatine phosphate the first "classical theory" was that the breakdown of glycogen to lactic acid furnished the energy for contraction. Lundsgaard (1) had shown that creatine phosphate is broken down into creatine and phosphate in muscle tissue poisoned with iodoacetic acid without the formation of lactic acid in muscular contraction. It was supposed that the energy for the contraction was supplied by the breakdown of the creatine phosphate. In the presence of oxygen the phosphoric acid and creatine are resynthesized to creatine phosphate and in a muscle deprived of oxygen the resynthesis is incomplete. The formation of lactic acid after the contraction was over was believed by Lundsgaard to supply the energy for the resynthesis of the creatine phosphate.

About this time Meyerhof and Lohmann (4) observed that at a pH from 8 to 9 the addition of adenosine triphosphate to muscle extract containing polysaccharide caused a formation of creatine phosphate and adenylic acid. They believed that this reaction was the immediate source of the energy for the resynthesis of creatine phosphate and that the later formation of lactic acid served to supply energy for the resynthesis of adenosine triphosphate. Lohmann (5) next observed that the phosphate interchange was reversible. At a pH of 7 adenylic acid reacted with creatine phosphate to form adenosine triphosphate and creatine. It was then postulated that the first reaction in muscular contraction was the breakdown of adenosine triphosphate and that in recovery this compound was resynthesized by creatine phosphate. Later, in the anaerobic recovery phase, the creatine phosphate

was resynthesized by the energy of the lactic acid formation and the final recovery was accomplished by oxidative reactions. Thus the second "classical theory" of the chemistry of muscular contraction may be formulated as follows:

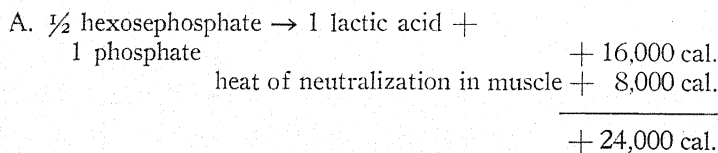
(1) Adenosine triphosphate \rightarrow Adenylic acid + $2\text{H}_3\text{PO}_4$
(Energy for contraction).

(2) Creatine phosphate \rightarrow Creatine + Phosphate (Energy for the resynthesis of adenosine triphosphate).

(3) Glycogen \rightarrow Lactic acid (Energy for the resynthesis of creatine phosphate).

(4) Oxidation of a small part of the lactic acid (Energy for the resynthesis of the remainder of lactic acid to glycogen).

Meyerhof (6) has listed the following reactions that occur in the anaerobic recovery of muscle:



B. Creatine phosphate \rightleftharpoons creatine + phosphate + 11,000 cal.

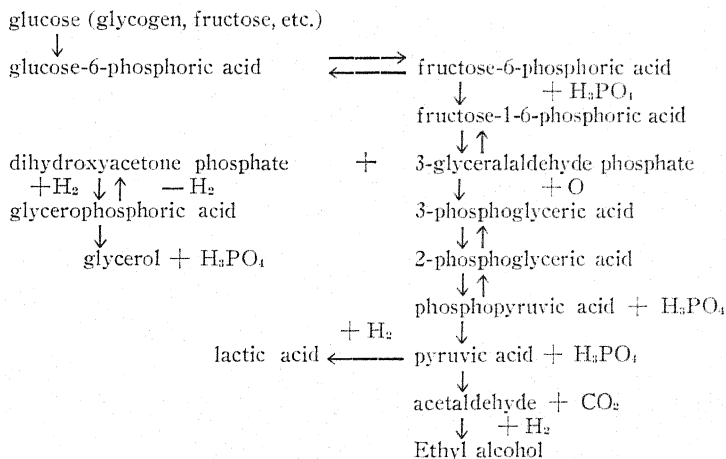
C. Adenylic acid + 2 creatine phosphate \rightleftharpoons adenosine triphosphate + 2 creatine + 0 cal.

D. Adenosine triphosphate \rightarrow adenylic acid + 2 phosphate + 24,000 cal.

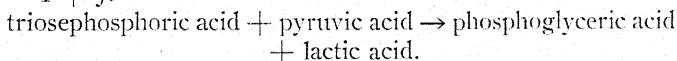
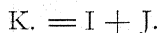
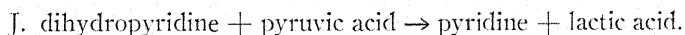
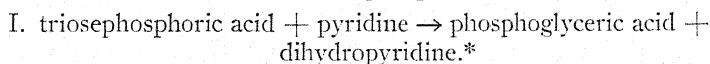
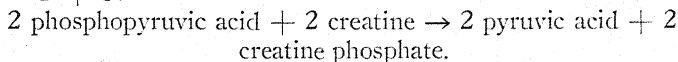
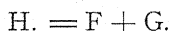
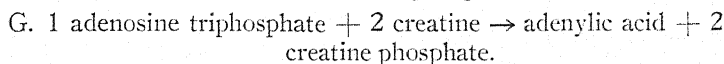
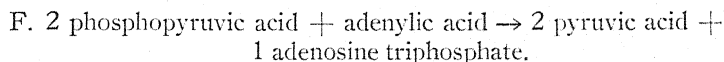
(Reactions C and D take place in the absence of carbohydrate breakdown.)

E. = C + D. 2 creatine phosphate \rightleftharpoons 2 creatine + 2 phosphate + 22,000 cal.

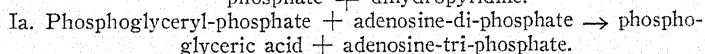
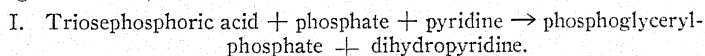
Breakdown of Carbohydrate



Parnas reaction



* Doctor Lipmann suggested that equation I, after Warburg's and Negelein's work, should be as follows:



L. triosephosphoric acid + pyridine + adenosine diphosphate + phosphate \rightarrow phosphoglyceric acid + dihydropyridine + adenosinetriphosphate.

M. adenosine triphosphate + creatine \rightarrow adenosine diphosphate + creatine phosphate.

N. = J + L + M.
triosephosphate + pyruvic acid + phosphate + creatine \rightarrow phosphoglyceric acid + lactic acid + creatine phosphate.

(Reaction N represents the synthesis of creatine phosphate during carbohydrate breakdown.)

TOTAL BALANCE
(After Meyerhof (6))

1 hexosephosphate + 4 creatine + 2 phosphate \rightarrow
2 lactic acid + 4 creatine phosphate.
32,000 cal. — 44,000 cal. = —12,000 cal.

Components	1 cc. of muscle extract contains	Ratio	Found	Calculated
	$\times 10^{-6}$ mol.			
A. Hexosediphosphate	—15.5			
B. Inorganic phosphate	—28.0	B/A	1.75	2
C. Creatine phosphate	+60.0	C/A	3.90	4
D. Lactic acid	+24.0	D/A	1.55	2

The transformations shown in reactions A to N above are accomplished by the action of enzymes. While all of these reactions have not been shown to occur in the living body they have been shown to occur in extracts of muscles containing the enzymes. Hence there is an excellent analogy presented by these reactions, and it is what one would expect to occur in the living muscle.

Ochoa (3) showed that in the presence of magnesium ions, inorganic phosphate, adenosine triphosphate, and cozymase, dilute rat brain extracts form lactic acid at a high rate from added glucose, hexose mono- or diphosphates. Dismutation between triose phosphate and pyruvic acid is markedly accelerated in the presence of a phosphate acceptor such as creatine phosphate or hexose diphosphate.

The first classical theory of muscular contraction, which

was discussed above, suggested that the production of lactic acid was the source of the energy of the contraction. This theory was then upset by Lundsgaard's discovery of the part played by creatine phosphate in the process. These results lead A. V. Hill (1) to publish his "Revolution in Muscle Physiology." As more and more experimental data are accumulated it is to be expected that a second revolution would soon break out. According to Sachs (1), whose article in *Physiological Reviews* may be said to announce the coming of this second revolution, the studies referred to above were conducted with frog muscle or muscle extracts. The development by H. A. and H. K. Davenport (1) of a technique of freezing the muscle tissue *in situ* with a mixture of powdered carbon dioxide snow and a volatile liquid, made it possible to study contraction of mammalian muscle with a normal circulation. In the first few seconds of tetani of varying durations using the Davenports' technique in rabbit muscle there is no breakdown of creatine phosphate, but large amounts of lactic acid and hexosemonophosphate were formed (Sachs and Sachs (2)). The phosphate groups for hexosephosphate formation were supplied by creatine phosphate. After longer stimulation hydrolysis of creatine phosphate occurred and the rate of increase of lactic acid and hexosemonophosphate diminished. After longer periods of stimulation there were decreases in lactic acid and hexosephosphate present and a resynthesis of creatine phosphate.

It was difficult to reconcile these findings with the Lundsgaard hypothesis. In the first place, in the initial period of contraction there was lactic acid formation without hydrolysis of creatine phosphate, whereas the reverse situation was to be expected, on account of the prolonged lag in anaerobic recovery. In the second place, the association of hydrolysis of creatine phosphate with lactic acid formation gave a reaction opposite to the requirements of the hypothesis. In the third place, the rapid accumulation of anaerobic metabolites did

not seem compatible with the idea that the contraction was anaerobic. In place of the older hypothesis Sachs (1) offered the following theory: "That the fundamental chemical reactions by which the energy for muscular contraction is supplied are oxidative, and that anaerobic reactions are used only when the supply of oxygen is inadequate. The principle anaerobic reaction is the formation of lactic acid from glycogen; whenever this mechanism becomes insufficient, the formation of hexosephosphate from glycogen and creatine phosphate is used as a supplementary source of energy. Implicit in this is the concept that hexosephosphate is not an intermediate in the formation of lactic acid. The primary function of creatine phosphate hydrolysis is that suggested by Fiske and Subbarow (1) to supply a base to neutralize lactic acid formed during contraction under deficient oxygen supply. Insofar as it is used to form hexosephosphate, it serves as a source of energy, but this is limited to a supplementary rôle, and to anaerobic reactions."

Sachs (1) continues to give a very interesting discussion of more recent work to support his hypothesis, especially in relation to the buffer theory of creatine phosphate hydrolysis in the neutralization of lactic acid. But Meyerhof (7) has replied to these observations and views of Sachs, as follows:

"It is clear that the difficulties already encountered with frog muscle in measuring the true velocity by chemical analysis of the muscle in different phases become insurmountable with warm blooded muscle at 37° C. For this purpose Sachs determined the resynthesis of creatine phosphate after 5 minutes of stimulation and after 5, 10, and 20 minutes of recovery. He makes the extrapolation that the velocity of resynthesis observed during this recovery was the same during the time of activity. But compare the curves of von Muralt (1) copied above (in Meyerhof's review) which show a very rapid anaerobic synthesis in the first seconds. In agreement with the new findings of D. K. Hill (1) who measured the oxidation at 0° C., Meyerhof and Nachmansohn (8) stated that the true velocity of the aerobic synthesis

of creatine phosphate after long activity could be measured in the isolated muscle by chemical means only at 0.5° C., the diffusion of oxygen interfering at higher temperatures. Assuming a temperature coefficient of 2.5 for 10° C. the synthesis at 37° C. would be complete in 1 minute instead of 40 minutes at 0.5° C.

"But the corresponding time in warm blooded muscle may even be much smaller since the velocity of oxidation depends, at the same temperature, according to D. K. Hill, on the amounts of cytochrome and cytochrome oxidase, which are appreciably higher in warm blooded muscle. The slow reactivity of the frog muscle is indeed of extreme advantage for the accurate analysis and the separation of the different phases of activity. In overlooking this technical point, and by uncritical application of the methods used here (at 0° C.) to warm blooded muscle (at 37° C.), a great confusion must necessarily arise.

"Now that D. K. Hill has demonstrated a complete agreement between the course of the oxidative restitution heat and oxygen uptake, the question appears to be finally settled in the sense of the 'classical view.'

"Kalckar (1) likewise does not agree with the views of Sachs since he states, 'It is at least obvious that Sachs is not justified in rejecting the Embden-Meyerhof scheme on the basis of his experiments.'"

Needham and Pillai (1) and Meyerhof, *et al.* (9) showed that half of the phosphate taken up in oxidations can be transferred to creatine. In the intact working muscle, however, the phosphate is not only transferred to creatine and sugar, but it is also liberated as inorganic phosphate, since muscular contraction is accompanied by an increase in inorganic phosphate and a decrease in creatine phosphate. Needham (2) stated that the break in the phosphate cycle very likely represents the transmission of the phosphorylation energy to the contractile system. In the dephosphorylation of adenylypyrophosphate (adenosine triphosphate) the free energy which is liberated is larger than that of most of the biological step reactions ($\Delta H = 11,000$ calories per phosphorus atom). It would seem that this pyrophosphate energy

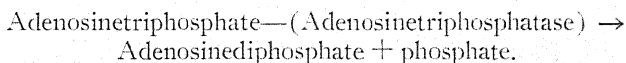
is used by the means of adenylypyrophosphate reacting with the contractile muscle protein, myosin. Lipmann (1) agrees with this view. Perlmann and Hermann (1) have shown, in this connection, that albumin and metaphosphate form on the acid side of the isoelectric point of the protein a crystalline compound which is soluble in dilute salt solutions. The metaphosphate was found linked to the basic groups of the protein. The loss of adenylypyrophosphate storage in the iodoacetic acid poisoning of muscle is accompanied by rigor (Lundsgaard (1, 4)). This would indicate that adenosine triphosphate dephosphorylation may be coupled to the relaxation (recharging) of the myosin system. Hence it is possible, according to Kalckar (1) that in the living cell adenosine triphosphoric acid is not dephosphorylated directly, but through cellular structures, acting as phosphate-transfer systems.

Myosin, the main protein component of muscular tissue, has occupied the attention of many investigators because of its extreme importance as the contractile element of living muscle. In addition there now exists the possibility that myosin is also an enzyme. Liubimova and Engelhardt (1) made the important discovery that adenosine triphosphatase activity was almost entirely associated with the myosin fraction of muscle and suggested that the enzyme might, in fact, be identified with myosin itself (Engelhardt and Liubimova (2)). Since the breakdown of adenosinetriphosphate is a reaction which is very closely associated with muscular contraction, and is also capable of supplying the energy for this process, it seems more than probable that the occurrence of the enzyme activity with the myosin fraction of muscle is of more than academic interest. Also the adenosinetriphosphatase activity of myosin has been observed by Needham (3), Edsall and Singher (personal communication with Doctor Bailey (1)), and by Szent-Györgyi and Banga (2).

Bailey (1) has recently extended the work of the Moscow investigators mentioned above. He showed that myosin prepared from skeletal and cardiac muscle tissue of various animals breaks down adenosinetriphosphate to adenosinediphosphate and that the reaction is activated by calcium and manganese. The activity is greatest at a pH of 9. While it is possible to obtain myosin free from adenosinetriphosphatase activity, it has not been possible to obtain the enzyme unassociated with myosin. In fact the enzyme and protein are considered by Bailey and others to be identical.

Bailey has correlated these new facts with the contraction process as follows:

- (a) Myosin and ATP-ase are identical.
- (b) Myosin is the contractile element disposed in the form of folded chains lying roughly parallel to the axis of the fiber.
- (c) The energy of contraction is provided by the reaction,



(d) Calcium is necessary for muscular contraction and it activates the enzyme.

(e) Contraction is also accompanied by ionic changes, chiefly by an increase in diffusible calcium (Weise (1)).

(f) The essential feature of excitation and contraction is the liberation of calcium in the neighborhood of the adenosinetriphosphatase grouping, which causes an instantaneous breakdown of $\text{ATP} \rightarrow \text{ADP} + \text{P}$, releasing a large amount of energy for the contraction process.

(g) Finally, the factor which limits the supply of energy to the contractile element is the rate of ATP resynthesis.

It is well known that many substances of the nature of the simple and polymerized nucleotides may enter into the structure of many conjugated proteins and the resulting enzymes

are of extreme importance in physiology. According to Bailey the contractility of muscle is due to changes in configuration of the polypeptide chain of myosin and adenosinetriphosphate shows a special type of affinity between certain groups of the protein and simple or polymerized ATP molecules.

From the above data it is evident that the work of Liubimova and Engelhardt, as well as that of Needham and Bailey, relating myosin and adenosinetriphosphatase activity together, is a most fundamental contribution to muscle biochemistry. (Cf. Review by Engelhardt, 27).

J. Needham, *et al.* (5) have recently published a further interesting account of the physico-chemical relationship between adenylypyrophosphate and myosin which today seems very important for any fundamental understanding of protein contractility. The behavior of myosin's flow-birefringence (FB), and relative viscosity (RV), at different pH levels is interesting. Just above pH 5.5, as the protein comes into solution above its isoelectric point, both FB and RV decline from high values to a constant level reached at about pH 6.3 which is maintained until a pH of 9 is reached at which the two properties begin to diverge. At pH 11, FB declines to zero while RV rises to values as high as they were just above the isoelectric point. They interpret this as disaggregation or tangle-formation perhaps accompanied by hydration. After the action of adenylypyrophosphate, FB invariably returns to its initial value. If samples of myosin, the FB of which has been reduced by various agents, are reprecipitated, the protein is found to be soluble, and the FB is largely restored.

When a myosin sol is treated with adenylypyrophosphate at concentrations as low as 0.004 M, the FB is reduced some 40 to 60 per cent, the anomaly is unaffected, and the RV is reduced by some 20 per cent. In 15 minutes to several hours both FB and RV return to their original values accompanied by a splitting off of inorganic phosphate from adenyly-

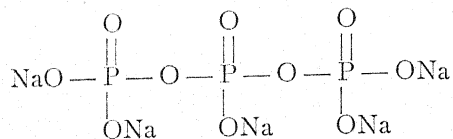
pyrophosphate. These changes can be repeated several times with the sample of myosin. The question immediately arises whether this represents a true contractility of the myosin particles. Since the anomaly is retained, the particles must continue to be rod-like, but since the FB is reduced, their axial ratio must have been less than before. At the same time changes of disaggregation and of hydration are possible. Even if the phenomenon were only one of disaggregation rather than true contraction, the physiological effect and significance are obvious.

Needham, *et al.* (5) from dosage data of the above experiments and assuming a molecular weight of 67,000 for myosin, calculated that 30 moles of adenylypyrophosphate are required for every mole of myosin, or one for every 19 amino acids in the myosin chain. Whether the fact that phenylalanine and methionine occur 16 times in the repeat unit, and tyrosine 18 times, may have any significance in this connection remains to be determined. But it is very probably true that adenylypyrophosphate, the actual energy-providing substrate of the contractile protein itself, may in fact be the agent of contraction, which would bring about its initial combination with the enzyme. Myosin is also considered to be the enzyme itself and the English workers speak of a "contractile enzyme" in this connection.

The viscosity data suggested a specificity in this action of adenylypyrophosphate on myosin. They tested 16 related and similar organic compounds which occur in muscle tissue with negative effects. The same was true after testing 5 or 6 inorganic muscle compounds.

They were, however, able to observe the adenylypyrophosphate effect when they used adenosine-diphosphate on *impure* myosin preparations. If the myosin is precipitated 3 times adenosine-diphosphate has no effect on it. This is due to the fact that Kalckar's "myokinase," which converts adenosine-

diphosphate partly into adenylypyrophosphate, is removed in the purification process. The adenylypyrophosphate effect was also obtained, even on the thrice precipitated myosin preparation, by inosinic (inosine-triphosphate)-pyrophosphate, where the amino group on the purine ring has been replaced by a keto group. Inorganic triphosphate was prepared,



which is hydrolyzed by muscle extracts. This substance is split by the enzyme giving inorganic phosphate, and, as in the case of adenylypyrophosphate and inosine-pyrophosphate, the process is accelerated by Ca^{++} ions, but it has no effect upon FB. It does, however, exert a certain degree of competitive inhibition on the adenylypyrophosphate reaction, diminishing the FB to 63 per cent of the control and the phosphorolysis to 40 per cent of the control.

It would appear, therefore, that myosin is a *specific triphosphatase*. Since triphosphate combines with myosin and is split without causing any fall in FB, it is possible that the purine-ribose end of the adenylypyrophosphate molecule is also of great importance in changing the shape of the myosin molecule.

The English workers conclude their interesting paper as follows:

"It now seems fair to say that the interaction between adenylypyrophosphatase and its natural substrate brings about deep-seated and optically reversible effects upon the enzyme's physical state. Whether these changes are micellar in character, or whether they are analogous to the configurational changes established for the keratins, remains as yet uncertain. Could adenylypyrophosphate itself then be normally *in vivo* the agent of con-

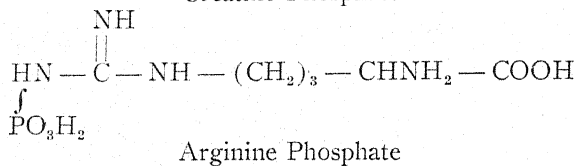
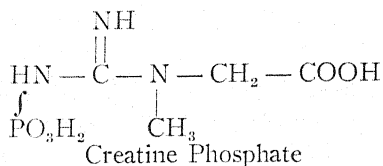
traction? Were this so, the reception of the nervous or other stimulus would essentially allow the enzyme and substrate to come in contact. By this enzyme-substrate combination the configuration of the contractile enzyme would itself immediately be changed (as pictured by Astbury and Dickinson (1), Astbury (2)) and the latter splitting off inorganic phosphate from the substrate would supply the energy needed for the relaxation and recharging of the myosin fibril. The solution of these problems will be of much importance, not only for muscle physiology, but also for many other fields of biology into which fibre-properties enter, such as morphology and morphogenesis (Cf. the recent work of Pollister (1) and Hobson, L. B. (1))."

Phosphate Bond Energy. Lipmann (1) has written an interesting review of this subject. The discovery of creatine phosphate and the rôle it plays in the contraction process early suggested an important relation of creatine to the energy supply of muscle. The biochemistry of the energy rich phosphate bond was introduced with this discovery. The discovery of creatine phosphate and its relation to the contraction process also caused a revision of the view that the breakdown of glycogen to lactic acid furnished the energy for this process. Lundsgaard (4) found that equal amounts of ultimate heat energy, irrespective of its origin from creatine phosphate or glycogen, did equal amounts of the same mechanical work. By this finding of Lundsgaard the application of phosphate bond energy for the driving force of the muscle machine was established.

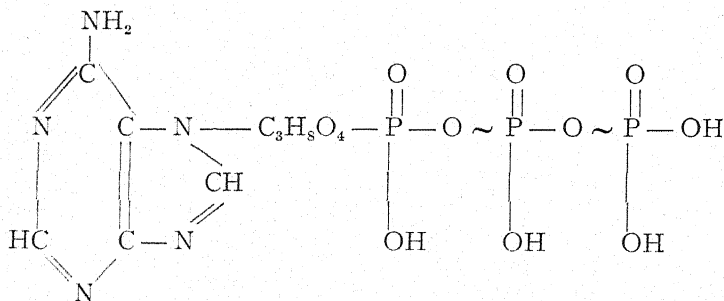
We may now reexamine the structures of several phosphate compounds in relation to phosphate bond energy. In three bond types the high group potential is caused by the anhydric nature of the bond. According to Lipmann, anhydization takes place either between two phosphates ($P-O \sim P$) or between a phosphate and a carboxyl ($O=C-O \sim P$) or an acid enol ($C=C-O \sim P$). In guanidine phosphates ($N \sim P$), such as creatine or arginine phosphates, the direct

connection between phosphorus and nitrogen is held responsible for the high group potential by Meyerhof and Lohmann (10).

The phosphate bond energy is located in creatine and arginine phosphates as follows:



In adenosine triphosphate or triphosphoric acid the energy rich bonds would be located between the oxygen and phosphorus, as follows:



Adenosine Triphosphoric Acid

In these compounds the energy rich bonds are designated between N and P and O and P by the symbol \sim .

According to Lipmann (1), reaction phases can be deter-

mined in the constantly occurring metabolic turnover of phosphate. These are: (1) introduction of inorganic phosphate into ester linkage, (2) the generation of energy rich bonds (\sim ph) by oxidation-reduction reactions, (3) the taking over and distribution of \sim ph and the regeneration of inorganic phosphate. For the maintenance of these complicated series of reactions there must be a well balanced equilibrium between the great number of enzymatic reactions involved. Removal of phosphate bond energy generated in oxidation-reduction reactions by adenylic acid occurs. A fine interplay between oxidation-reduction and phosphorylation-dephosphorylation results. Thus it is seen that phosphate enters into primary organic bonds over the pathway of oxidation-reduction reactions. Under these conditions phosphate bonds of high potential are formed by "transphosphorylation" and in the cells this high potential is maintained, or a fall in potential occurs with the formation of an ester bond.

The de- and rephosphorylation of adenylyl pyrophosphate bond energy is due to different apo-enzymes (protein parts) (Lohmann (11)). The adenosine polyphosphate/adenylic acid pair reacts reversibly with the pairs creatine + creatine phosphate (Lehmann (1)) and phosphoglycerate + phosphoglycerol phosphate (Warburg and Christian (1)).

The essential function of the metabolic group transfer is the maintenance of the group potential at a given level. In the transfer of phosphate groups between organic molecules the phosphate group never passes through the inorganic phosphate stage as was shown by the use of radioactive phosphate by Meyerhof, *et al.* (12) and Korzbyski and Parnas (1).

Anaerobic Metabolism. In anaerobic metabolism fermentation and glycolysis are reactions where the energy, derived partially from the catabolism of glucose, is converted almost entirely into phosphate bond energy. In oxidation-reduction reactions there are three phases: (1) the pre oxidation-reduc-

tion transformation period, (2) the oxidation-reduction reaction, and (3) the post oxidation-reduction period.

Pre Oxidation-Reduction Period. With glycogen the first phosphate is introduced by phosphorolysis. Introduction of the second phosphorus from adenylic acid with the formation of the fructose-diphosphate is shown in muscle and yeast extracts. In these series of reactions the hexose is broken up eventually into two parts and the final product here is phosphoglyceraldehyde.

Post Oxidation-Reduction Period. The reactions, according to Lipmann (1) in this period are as follows: $\text{ph-3-glyceryl} \sim \text{ph} \rightarrow \text{ph-3-glycerate} \rightleftharpoons \text{ph-2-glycerate} \rightleftharpoons \text{pyruvic enol} \sim \text{ph} \rightarrow \text{pyruvate}$.

The phosphate bond energy ($\sim \text{ph}$) generated in the oxidation-reduction reaction is removed from the carboxyl of phosphoglyceric acid with the conservation of the bond energy through adenylic acid. It finally appears as in muscle as creatine phosphate bond energy. The pre oxidation period is mostly energy consuming while the post oxidation-reduction period and the oxidation-reduction reaction proper yield most of the energy. This is then drawn off from the anaerobic cycle as phosphate bond energy. Lundsgaard (3) observed that up to 1.9 mole of energy rich phosphate bonds, determined as creatine phosphate, is equivalent to 1 mole of lactic acid formed from one-half mole of glucose.

The Oxidation-Reduction Reaction. The hydrogen in lactic acid and in alcoholic fermentations is carried from reductant to oxidant by the cozymase, or diphosphopyridine dinucleotide. The pyridine combines with two independent enzyme systems for the partial reactions of hydrogenation and dehydrogenation. Thus a comparison of hydrogen and phosphate transfer is seen to be analogous to the enzymatic process. The independence of donator and acceptor systems in almost all biochemical transfer reactions is a fact of much

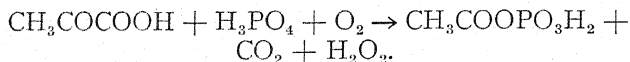
importance in metabolism. The cell is able to interchange acceptor and donator systems under different conditions.

When glucose splits to 2 lactic acids, 4 phosphate bond energies are made available by the formation of 2 phosphate bond energies in the oxidation-reduction and 2 enol-phosphates by dehydration of phosphoglyceric acid. If glycogen is the substrate, 3 and possibly 4, of the phosphate bond energies remain available. Needham and Pillai (1) then showed that almost 4 moles of creatine phosphate could be reformed per mole of glycogen \rightarrow glucose metabolized in the recovery phase of muscle activity. With glucose, however, where 2 of the phosphate bond energies are used for primary phosphorylation, only 2 of the 4 phosphate bond energies remain available. Lipmann (1) calculated that the change in free energy with decomposition of an energy-rich phosphate bond amounts to $-10,000$ cal. The $40,000$ cal., represented by the 4 energy-rich phosphate bonds, can then be compared to the total change in free energy with the decomposition of one mole of glucose (glycogen) to 2 moles of lactic acid. For this reaction Burk (1) calculated $\Delta F = \text{ca. } -58,000$ cal. *Hence up to 70 per cent of the total energy may be converted into phosphate bond energy.*

The breakdown of creatine phosphate, or more recently, adenylyl pyrophosphate bond energy, are believed to be the reactions closest to muscular activity. Lipmann (1), however, states that no decision can be made as to where the phosphate bond energy is taken off to operate contraction. He believes that muscular contraction is quite probably driven by energy derived from both anaerobically and aerobically generated phosphate bonds.

Aerobic Metabolism. This subject has been thoroughly reviewed by Lipmann (1) in regard to phosphate bond energy. He showed (2) the connection between pyruvate oxidation and phosphate turnover in the simple oxidation of *Bacterium*

Delbrueckii. This oxidation stops after removal of 2 hydrogens from pyruvic acid. It was observed that (a) pyruvate is only dehydrogenated in presence of inorganic phosphate, (b) pyruvate dehydrogenation generates phosphate bond energy (causes phosphorylation of adenylic acid), (c) the primary oxidation product contains phosphate and behaves like acetyl phosphate and synthetic acetyl phosphate transfers phosphate bond energy to adenylic acid. He pictures the dehydrogenation as follows:



Johnson (1) has published an interesting discussion of the relation of aerobic phosphorylation in the Pasteur effect. The various mechanisms which have been suggested to explain the Pasteur effect have also been summarized by Burk (2). Johnson's mechanism of this effect is summarized as follows: "If both anaerobic and aerobic carbohydrate breakdown are necessarily phosphorylative processes, inorganic phosphate and a phosphate acceptor are essential reactants; in their absence neither glycolysis or oxidation could proceed. The Pasteur effect could then be regarded as the removal of glycolysis which takes place when concentrations of inorganic phosphate and phosphate acceptors becomes low because of the phosphorylative oxidations which occur in the presence of oxygen."

Five conditions are necessary for the operation of this mechanism:

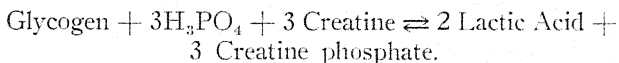
- (1) The glycolytic reactions must be reversible.
- (2) Phosphorylation (esterification of inorganic phosphate) must be an essential step in both glycolysis and oxidative processes.
- (3) The oxidative phosphorylation reactions must be

capable of reducing the inorganic phosphate (and phosphate acceptor) concentration to a level lower than that attained at glycolytic equilibrium.

(4) The number of molecules of phosphoric acid esterified when 1 mole of carbohydrate is oxidized must be greater than the number esterified when 1 carbohydrate molecule is glycolyzed.

(5) The same reservoirs of phosphate ester, inorganic phosphate and phosphate acceptor, must be available to both glycolytic and oxidative enzyme systems.

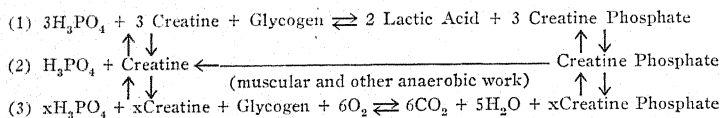
Johnson (1) summarizes the glycolytic reaction as follows:



Whether appreciable reversal can occur under physiological conditions depends upon the equilibrium point. That the equilibrium falls (as in muscle glycolysis) well within the physiological range of reactant concentrations is shown by Lundsgaard's (3) data. He shows that after muscular work glycolysis proceeds only to a certain definite equilibrium point. High concentrations of lactate occur only with high concentrations of creatine and inorganic phosphate and low concentrations of creatine phosphate. High concentrations of creatine phosphate and low inorganic phosphate concentrations permit the formation of only a limited amount of lactic acid.

It has also long been known that phosphorylation is a necessary component of the glycolytic process. In aerobic recovery of muscle phosphorylation likewise occurs. Meyerhof and Nachmansohn (8) showed that the oxygen uptake which resulted when oxygen was admitted to fatigued muscle brought about an amount of creatine phosphate resynthesis

Upon these assumptions Johnson (1) gives the following diagram to illustrate the reactions occurring in isolated muscle tissue:



A part of the energy of carbohydrate oxidation is converted to the energy of phosphorylation by the aerobic phosphorylation mechanism. The energy of phosphorylation is then expended by the resynthesis of glycogen from lactic acid. In a

steady state of rest or low work output under aerobic conditions glycolysis would be regulated by the fact that aerobic phosphorylations can proceed at a lower concentration of inorganic phosphate than is compatible with an appreciable glycolysis rate. Also the rate of carbohydrate oxidation will be limited by the available concentration of inorganic phosphate. It would then follow that oxidation is slow in the resting state due to a lack of inorganic phosphate which is essential to the oxidative phosphorylation process. The liberation of phosphate during metabolic work permits increased carbohydrate oxidation. When the rate of inorganic phosphate liberation exceeds the rate of oxidative phosphorylation the resulting accumulation of phosphate will accelerate glycolysis.

In view of these considerations Johnson considers that the mechanism of the Pasteur effect is readily outlined. Since oxidative phosphorylation is more energetic, *i.e.*, capable of attaining a higher creatine phosphate-creatine ratio than glycolytic phosphorylation, the addition of oxygen to muscle rapidly reduces the level of inorganic phosphate and raises the creatine phosphate-creatine ratio until a point is reached where glycolysis is reversed. Since the number of moles of phosphoric acid esterified per mole of carbohydrate consumed is much larger in oxidation than in glycolysis, a much lower rate of sugar disappearance suffices to maintain a high phosphorylation level in the face of the energy demands for muscular work or other energy producing reactions. A measure of the Pasteur effect is the number of sugar molecules protected from glycolysis per mole of sugar oxidized. This quotient should be equal to the ratio of the number of moles of phosphoric acid esterified when a carbohydrate molecule is oxidized to the number esterified when a carbohydrate molecule is glycolyzed. The Pasteur effect in yeast and muscle would be interpreted as following from the fact that, aerobi-

cally, a relative low rate of sugar utilization suffices to re-esterify phosphate as rapidly as it is liberated in energy consuming reactions. Colowick, *et al.* (3) have recently shown that at least 10 atoms of phosphate are esterified per mole of glucose oxidized. Cori (4) subscribes to the view that the energy of aerobic phosphorylation is utilized in carbohydrate resynthesis.

Phosphorylation and Respiration. Kalckar (1) has recently reviewed this subject. Lennerstrand and Runnstroem (1) observed a relation (coupling) between phosphorylation in dry yeast preparations and respiration and also observed that triose phosphate was oxidized to phosphoglyceric acid. Lipmann (3) showed that dehydrogenation of pyruvate by *Bacterium Delbrueckii* was a non-fermentative reaction and that it was coupled with phosphorylation. Phosphorylations coupled with respiration have also been observed in animal tissues. This was shown by Kalckar (3) in minced kidney cortex and extract from kidney cortex. These systems shaken with oxygen show an intensive respiration. If phosphate and glucose are present and fluoride is added in order to stop dephosphorylation, most or all of the inorganic phosphate disappears in 15 minutes. The phosphoric ester which accumulates is mainly fructose-1-6-diphosphate. Fructose-6-phosphate added is phosphorylated to fructose-1-6-diphosphate. A new kind of esterification, which is so far only observed in kidney cortex, is the rapid phosphorylation of glycerol in kidney extracts; the ester formed was 1- α -glycerophosphate. Adenylic acid is phosphorylated to adenylypyrophosphate very rapidly. These phosphorylations depend upon the rate of respiration. An inhibition of respiration by cyanide also inhibits phosphorylation. Phloridizin, however, inhibits phosphorylation much more than respiration. Addition of 5 mg. of citric acid or a dicarboxylic acid, such as glutamic acid, approximately doubles the respiration with an even larger

increase in the phosphorylation of sugars, glycerol or adenylic acid.

The results of the studies of Colowick, *et al.* (2) showed that the coupled phosphorylation system needs Mg ion, succinic acid, adenylic acid and pyridine nucleotide (cozymase). It was also shown that in experiments with kidney and heart extracts, the oxidation of citric and glutamic acids gave considerably more phosphorylation than the oxidation of succinate. In the studies with heart extract the oxidation of pyruvate gave high yields of phosphorylation. Per millimole of oxygen consumed 4 millimoles of phosphate were taken up.

Belitzer and Tzibakova (1) and Banga, *et al.* (1) have observed even higher ratios of P/O_2 and these investigators stated that the high ratio of phosphorylation to respiration can be explained by the uptake of phosphate when hydrogen passes from one hydrogen transfer system to another. In this passage from one transfer system to another energy is provided for phosphorylations. The studies of Colowick, *et al.* (3) showed a quantitative relation between combustion of glucose (complete oxidation to CO_2) and phosphorylation. For every mole of glucose oxidized to CO_2 an additional 5 or 6 moles of glucose disappear, 5 moles of which are accumulated as fructose-di-phosphate. Probably 10, if not 12, steps in glucose combustion can give rise to phosphate uptake.

Belitzer (2) has recently demonstrated a marked stimulation of the respiration of muscle pulp by creatine, during which creatine is phosphorylated to creatine phosphate. The increase in respiration by creatine is determined by its capacity to accept phosphate groups. This is the first demonstration of a compulsory coupling between respiration and phosphorylation in animal tissues. In 1937 Belitzer, *et al.* (3, 5) showed that an increased respiration of isolated frog muscle causes a direct increase in the creatine phosphate

content of the muscle. In anaerobiosis of muscle all or most all of the lactic acid formed as an intermediate is consumed and the resynthesis of glycogen is low or does not occur at all, even though an exchange between adenosine triphosphoric acid and creatine phosphate occurs. It was concluded that the fundamental cycle in resting muscle under aerobic conditions is not glycolysis and resynthesis of glycogen, but the splitting of the creatine phosphate-adenosine triphosphoric acid system and its oxidative resynthesis.

Tzibakova (9) showed that sodium selenite, copper salts and phlorhidzin inhibited the synthesis of creatine phosphate although respiration was affected to a lesser degree. In the oxidation of glutamic acid by cardiac muscle creatine phosphate is formed simultaneously from creatine and inorganic phosphate. The addition of arsenite inhibits both processes. Belitzer and Tzibakova (1) showed that the synthesis of creatine phosphate from creatine by washed muscle tissue from the pigeon or rabbit occurs rapidly at a pH above 7 provided the presence of dehydrogenation substances, such as pyruvic, citric, malic, succinic, acids, etc., are present in the system. Adenylic acid is far less important as a phosphorylating agent in muscle than is creatine. Iodoacetic acid inhibits respiration due to an inhibition of glycolysis, but it does not inhibit the phosphorylation of creatine. Creatine phosphate participates in each step of the oxidation of glucose. It was estimated that more than 30 moles of creatine phosphate are synthesized during the oxidation of 1 mole of glucose to CO_2 and water.

The above discussion will show that much progress has been made in determining the relation of creatine to carbohydrate, potassium, and phosphate metabolism. This relation is a very complicated one and further study will possibly serve to alter previous theories in the field. The relation of phosphate bond energy (which involves chiefly creatine phos-

phate), oxidation-reduction reactions and the relation of phosphorylations to respiration, will be, according to Kalckar, one of the major problems of the future. In the words of Szent-Györgyi (1) "whatever the mechanisms of the energy transfer of the electron transport may be, the importance of phosphoric acid esters, in the problem of biological energetics, admits of little doubt. The discovery of this energetic significance of phosphate is one of the most important and striking discoveries of modern biological research which, I expect, will, in the future, open unforeseen vistas, and will bring us closer to the understanding of life."

At the recent symposium (26) on respiratory enzymes held at the University of Wisconsin, Cori, Kalckar, Lipmann, Meyerhof and Johnson discussed the mechanism of phosphorylation in carbohydrate metabolism. Cori stated that the coupling between respiration and phosphorylation has given an insight into the mechanism of energy transfer in the cell. Mechanisms exist in the cell which regulate the direction of individual enzymatic reactions and which lead to a high degree of integration of overall effects. The next approach, according to Cori, is to study the mechanisms underlying this regulatory function.

CHAPTER XV

FUEL OF MUSCULAR EXERCISE. EFFECT OF MUSCULAR EXERCISE ON CREATINE-CREATININE EXCRETION. EFFECT OF INGESTION OF AMINO ACIDS, PROTEINS, PHOSPHATES, VITAMINS, CREATINE AND CREATININE UPON HUMAN ENERGY OUTPUT. RÔLE OF GLYCINE IN METABOLISM

The Fuel of Muscular Exercise. It is well known that, following the work of Hill and Meyerhof, the fuel of muscular work was considered to be chiefly carbohydrate. In recent years, however, physiologists have become acquainted with the fact that fat can also furnish the energy for muscular work. Hence at the present time the oxidation of both sugar and fat is considered to furnish the fuel for muscular work. This subject has been reviewed by Rapport (42) and Dill (2).

What rôle does protein play in this process? It will be recalled that many years ago Liebig stated that the breakdown of protein required in turn a replacement that could only be furnished by a diet rich in protein. Fick and Wislencius, however, attempted to refute this idea. They showed that the output of nitrogen in the urine for 24 hours was not increased by climbing the Faulhorn to a height of almost 2,000

meters. Then the idea became general that there was no breakdown of protein during muscular exercise. Whether this view can be accepted at the present time is doubtful. It should be remembered that muscle tissue is unusually rich in protein and there is some evidence that the protein, myosin, may be linked to creatine phosphate, which would then make it play a leading rôle in the chemistry of muscular contraction. It is also well known that individuals doing heavy muscular work require large amounts of protein in their diets.

It is also a debatable question whether or not the urinary nitrogen is a good indicator of what is happening to protein metabolism in the body during exercise. It is now known that many exchanges of nitrogen from one amino acid to another and transformations of the amino acids into protein and back again may occur with little change in the urinary nitrogen content. Cathcart (2) reviewed the subject and did find small, but definite, increases in the nitrogen output during exercise and he believes that protein can also be used to furnish some of the energy for muscular work. Peczenik (1) observed that the activity of mice after being fed on diets rich in protein was much better than on a fat or one sided diet. The recent work on protein and amino acid feeding to be discussed below will also show that protein should be considered effective in this connection. The writer feels that, at the present time, it is safe to conclude that protein, fat and carbohydrate can each contribute to supply energy for muscular work.

Gemmill (1) has written the most recent review of the subject. From a survey of the literature he concludes that carbohydrate is of primary importance as a fuel for muscular exercise. Protein is changed to carbohydrate, and is able to furnish fuel for exercise in this manner. (This statement ignores the effect of proteins and amino acids upon increasing the energy output due to the increased formation of

creatine phosphate.) Fat is not directly utilized by the muscles. But the indirect utilization of fat and protein is an efficient process.

Effect of Muscular Exercise on Creatine-Creatinine Excretion. The bulk of the evidence in the literature shows that moderate exercise does not serve to increase the creatine-creatinine excretion, except occasionally. This result is to be expected since there is only a small amount of free creatine present in the muscles above that combined as creatine phosphate, and since creatine does not increase creatinine excretion in the urine. About 25 per cent more creatine than is usually present can be stored for a short time in the muscles and even if the resynthesis of creatine phosphate should not be complete after muscular exercise the extra creatine could easily be stored or oxidized in the muscles and not excreted.

Most of the recent evidence shows that training or exercise does not increase the creatine content of the muscles or the creatine excretion in the urine (Hobson (1); Ramiah, *et al.* (1); von Krüger (2); McClintoch, *et al.* (1); Lakhno (1); Mezinseco (1); Klimenko, *et al.* (1); Margaria and Foa (1)). On the other hand blood creatine increased in 60 per cent of the cases of muscular fatigue and creatinuria was observed in 90 per cent by Olman, *et al.* (1). Creatinine excretion increased parallel with the total nitrogen in working conditions in a hot climate (Ehrismann (1)). Eimer (4) showed that during bed rest the excretion of creatinine was lowered. Activity caused a marked increase in creatinine excretion. Palladin and Okhrimenko (5) stated that the tail muscles of the fish contained more creatine than those of the back. Natural training served to increase the creatine content of fish muscle.

Dubuisson (1) studied the chemical processes which occur in smooth muscle before, during and after contraction. Four successive changes in reaction occurred.

1. *Alkaline phase* (before contraction): Maximum at the outset of period of increasing tension.

2. *Acid phase* (before contraction): Maximum toward the middle of period of rising tension. Probably an expression of the decomposition and resynthesis of adenylypyrophosphoric acid.

3. *Alkaline phase*: Begins during period of rising tension. Maximum at the onset of relaxation. Represents the hydrolysis and synthesis of creatine phosphate.

4. *Acid phase*: Begins during contraction. Maximum several minutes later. An expression of the formation of lactic acid.

These four stages also occur in striated muscle, but they are more difficult to measure due to the rapidity of contraction.

The Effect of Ingesting Various Substances upon Work Output. Like most scientific questions there is at present a difference of opinion as to whether the feeding of gelatin or glycine will increase the energy output in man. The results of negative studies in this connection are listed in Table 36.

TABLE 36

NEGATIVE RESULTS UPON HUMAN ENERGY OUTPUT
AFTER INGESTING GELATIN OR GLYCINE

No. of Subjects	Substance Ingested	Sex	Amount Ingested Per Day,	Author
			gm.	
6	Gelatin	F	60	Hellebrandt, <i>et al.</i> (1)
2	Glycine	M	15	Maison (1)
2	Gelatin	M	60	Maison (1)
9	Gelatin	M	60	Robinson and Harmon (1)
11 (rats)	Gelatin		½	Knowlton (2)
	Gelatin	M		Karpovich and Pestrecov (1)
8	Glycine	M	6	Horvath, <i>et al.</i> (2)
33	Glycine	M	4.5 to 6	King, <i>et al.</i> (1)

Several criticisms of the techniques used by these investigators will be made here. In the first place no analogy should be drawn from negative results in relation to positive results. If five individuals in one test show no increased energy output after ingestion of glycine or gelatin, this is no reason why five different individuals under other circumstances should not show increases in energy output under these conditions. Each individual should be considered alone without regard to results for other individuals. From the writer's experience, to be discussed below, only 75 per cent of a given number of individuals will show increases in energy output with the other 25 per cent showing nothing after glycine, or glycine-urea ingestion. *This is a normal physiological variation between individuals and should be recognized as such.*

In the second place several of the above tests were designed to show a training effect and under these circumstances no substance should be expected to show increases in energy output. This criticism applies to the studies of Hellebrandt, *et al.* (1), Robinson and Harmon (1), Karpovich and Pestrecov (1), Maison (1), and King, *et al.* (1). In the experiments of Karpovich the prisoners were paid by the hour for working on the bicycle ergometer and under these conditions they would strive to work as long as possible and nothing but a training effect could be observed. It is also very difficult to understand how his subjects did not know they were ingesting gelatin. In the tests of Maison only one muscle was tested. In Horvath's experiments (2) not enough glycine was given and the studies did not last long enough for effects to be shown. In Robinson's study a restricted protein diet was fed. We have repeatedly shown that glycine will not form creatine unless a normal or high protein diet is fed at the same time. If the subject is not receiving enough nitrogen to meet the daily demands for protein synthesis, then glycine,

or the amino acids hydrolyzed from gelatin, will go *first* to meet this demand, with the result that no creatine formation occurs and no increased energy output will be seen. On the other hand in at least 10 of our publications we have stressed the fact *that a diet normal or one rich in protein should be fed to animals and man if increases in creatine formation and in energy output are to be observed*. Neglect of this fact is the reason for some of the negative reports in the literature in studies of energy output and also in studies of clinical results in the myopathies. In the studies of Robinson and Horvath no increased creatine or creatinine formation was observed, which again confirms our view expressed herewith. On account of the wide publicity given to positive results with gelatin the writer predicts that many more negative results with this protein will be published.

Another criticism of gelatin feeding experiments is that the observers always take for granted that the gelatin is completely digested and absorbed. This may not always be the case, and cases of muscular dystrophy have been cured by simply adding a pancreatic digest to the food ingested. It is the conclusion of the writer that many of the negative studies reported above were incorrectly performed and need repetition using more carefully controlled conditions before negative results should be accepted. In the studies where the training effects were so prominent it is difficult to understand why the investigators should expect any additional substance to increase the energy output under these conditions. The maximum of physiological endurance for any individual cannot be exceeded by any means, and there is no point in an individual who is perfectly normal ingesting any substance to make him "supernormal."

King, *et al.* (1) have recently reported their results with glycine in increasing the work capacity of human subjects.

Since this study was conducted by officials of the Food and Drug Administration the "official" nature of the findings call for some comment. Four groups of *football* players were used as experimental subjects. They were all fed the same *diet* which was not mentioned. All were given physical examinations and found to be "*physically fit to perform arduous physical exercise.*" Glycine tablets alternated with placebo tablets (lactose and saccharose) were given. The training period was 13 days on the bicycle ergometer. The mechanical efficiency of the machine was not determined. The rate of work was about 144 watts per second. This statement has little meaning since the *load* against which the subject worked was not mentioned. There was no effect of training during the first three weeks. For the next three weeks two groups received only 4 to 6 gm. of glycine daily, and the other two groups received the placebo tablets. "A rapid and progressive increase in work output was observed for all groups regardless of the medication given." But King, *et al.* told the subjects that the placebo tablets contained gelatin, which they knew would give increases in energy output. It is, therefore, very certain that, since there was no training effect in the control periods, and since it is well known that the small amount of sugar given to two of the groups would not serve to increase energy output, that the increases in energy output of their groups A and C receiving placebo tablets, was due to *mental suggestion* and not to sugar. We believe that the increases in energy output in Groups B and D receiving glycine was due to glycine, and King, *et al.* cannot prove that this was not so. When these groups were placed on placebo tablets at the end of three weeks they maintained their energy output so King draws the conclusion that glycine does not increase energy output. Furthermore 6 gm. of glycine is a small daily dose. If they had given 12 gm. daily the energy

output would probably have been doubled (Beard (31, 36)). Furthermore the glycine tests lasted only three weeks. In our work it required three weeks for the first increases in energy output to occur. We maintain that they did not allow a sufficient time for the maximum increases in energy output to manifest themselves.

King, *et al.* (1) arrived at the following conclusions from their study:

1. "Thirty-three males of both white and negro races were given amino acetic acid in daily amounts from 4.5 to 6 gm., under rigidly controlled conditions, over a period of three weeks. There was no effect on the work capacity of these subjects when compared with controls receiving lactose-saccharose under identical conditions or with cross control of the same subjects when receiving lactose-saccharose in the same manner."

2. "A review of recent studies in protein metabolism reveals that the claims made for the especial value of amino acetic acid or gelatin in the treatment of fatigue or increasing endurance are unfounded on theoretical grounds and that protein in the ordinary diet is fully capable of supplying the amino acetic acid requirements of man. It is also pointed out that the incomplete protein gelatin is deficient in methionine, one of the amino acids necessary for the synthesis of creatine."

3. "It is evident from these experiments that a study of the effects of a drug on fatigue must be meticulously controlled: the drug under study should be administered alternately with placebo identical in physical characteristics and the identity of each should be unknown to the subjects as well as the investigators."

Results under 1. above have already been criticized. Under 2. the following criticisms are much in order:

- (a) The writer has also reviewed the literature on protein

metabolism and the statement that "glycine or gelatin does not increase the energy output" is unjustified and certainly not in agreement with the facts as will be shown in the discussion below. It seems strange that King, *et al.* should deny the possibilities of gelatin when they did not even test it out themselves. Such theoretical and official opinions, without scientific data to back them up, will hardly be accepted by those who are convinced that gelatin and glycine do increase the energy output. If the protein of the diet is capable of furnishing the glycine requirements of the body then why is it that many thousands of individuals suffer from weakness and fatigue "myasthenia mitis" and why are they able to increase their resistance to this weakness and fatigue when glycine is ingested? This fact is well known to the medical profession.

Since gelatin does not contain methionine it was concluded that it could not form creatine. (Barenstein (1), however, stated that this protein contains 0.97 per cent methionine.) It was assumed by this statement that methionine is specific and necessary for creatine formation. There is no evidence to back up this statement. About 20 different amino acids besides methionine can increase muscle creatine. Furthermore several investigators, including ourselves, have shown that gelatin can increase glycoamine or creatine formation and excretion (*Cf.* Chapters V and VI). Reference was made to Sachs' view that creatine is not used to increase the energy for muscular contraction. However, Meyerhof and Kalckar do not accept Sachs' views. Sachs' results were obtained working at 37° C. and Meyerhof stated that at this temperature it was impossible to obtain accurate results because of the speed of metabolic reactions. On the other hand most of the earlier observations of Meyerhof and others were performed at 0.5° C. at which temperature these reactions are

much slower and can be measured with a fair degree of accuracy.

Under 3. glycine is referred to as a drug. Dorland's medical dictionary defines a drug as a "crude medicinal substance" which probably has little meaning. On the other hand most individuals consider glycine as a food product as it is a protein derivative and exerts physiological and chemical properties rather than pharmacological properties in the body.

In a recalculation of Doctor King's experiments there appears to be an error in the rate at which his men worked and also in the total amount of work which they produced. King reported that his subjects worked at 144 watts *per second* which, according to the following calculation, would show that the subjects were working at 11.6 Horsepower, an absolutely impossible rate. For example,

$$\begin{aligned} 144 \text{ watts per second} &= \text{rate of work} \\ 44.24 \text{ foot lbs. per minute} &= 1 \text{ watt} \\ 33,000 \text{ foot lbs. per minute} &= 1 \text{ H.P.} \end{aligned}$$

Then $144 \times 60 \times 44.24 = 382,233$ foot lbs. per minute, and
 $\frac{382,233}{33,000} = 11.6$ Horsepower. On comparing King's results

with those of others it would appear that he should have reported in watts per minute, which would account for the rate of work being about 50 or 60 times too great.

Another error is apparent in the figures for the total work output as given in King's charts. They do not agree with the erroneous rate of 144 watts per second as discussed above, but are still further out of line according to the following calculations:

160 Kilowatt seconds from Chart I, Group B, consisting of 8 men during a five minute work period (maximum reported); $44.24 \text{ ft. lbs. per minute} = 1 \text{ watt}$.

Then

$160 \times 1,000 \times 60 \times 44.24 = 424,704,000$ foot lbs. per minute.

$$\frac{424,704,000}{8} = 53,088,000 \text{ foot lbs. per man average.}$$

$$\frac{53,088,000}{5} = 10,617,600 \text{ foot lbs. per man per minute.}$$

$$\frac{10,617,600}{33,000} = 322 \text{ Horsepower rate of work per man, average.}$$

Apparently in multiplying through for getting total work output for the group Doctor King has kept designations of watt seconds whereas he has really converted to total watts. Thus, to take a typical case, say 144 watts per second was the rate of work, with 8 men in the group working for a 5 minute period. Then, $144 \times 60 \times 5 \times 8 = 345,600$ total
 345,600
 watts performed by the group. Then $\frac{345,600}{1,000} = 345$ Kilo-

watts (which is in the approximate range of the charted results in "Kilowatt seconds").

The author concludes that the studies of King, *et al.* (1) were not conducted in such a way as to show that glycine would or would not increase the energy output; the amounts of glycine ingested were too small and the time (3 weeks) too short to obtain results. It is also very unlikely that their results will be accepted by those individuals who are convinced that glycine does increase the energy output.

That gelatin ingestion will increase the energy output has been shown by Ray, *et al.* (1) and Kaczmarek (1, 2, 3). Ray studied the effect of gelatin ingestion on muscular fatigue in 6 men and 4 women. There was no effect in the women. There were increases in watt output from 37 to 240 per cent in the men subjects as compared to the maximum produced

during the training level. In his first study Kaczmarek (1) observed the following effects:

Ten athletes increased their daily average work output by 216 per cent while supplementing their diet with 1.5 ounces of gelatin daily. Under similar conditions the non-athletes showed an improved work output of 52 per cent during the gelatin period. There was an inverse relationship of the rate of work and its summation and this shows the necessity of working at a constant rate for purposes of comparing the work output. In the 12 girls the increase in the training period was 26 per cent as compared to 501 per cent in the gelatin periods. In the second post-gelatin period there was a drop of 465 per cent in the work output.

Kaczmarek (2, 3) in later studies also measured the work output, heart and pulse rates during sham feeding and gelatin feeding. Two groups of 6 men each were used. His results are listed in Table 37.

TABLE 37
EFFECT OF GELATIN INGESTION ON HUMAN
ENERGY OUTPUT
(After Kaczmarek (2, 3))

GROUP A					
Period	Training, Per Cent	Gelatin (1), Per Cent	Gelatin (2), Per Cent	Sham Feeding, Per Cent	Gelatin (3), Per Cent
Work	75	379	279	-351	970
Pulse	96	528	403	-463	1,557
GROUP B					
Period	Training, Per Cent	Gelatin (1), Per Cent	Post- Gelatin, Per Cent	Sham Feeding, Per Cent	Gelatin (2), Per Cent
Work	8.5	304.4	-3.6	-116.9	531.1
Pulse	12.0	362.6	+57.0	-137.1	611.5

From these results it is clearly seen that training increased the work output in the subjects in Group A by 75 per cent.

The first gelatin period increased it by 304 per cent in excess of the training maximum. In the second gelatin feeding period the training maximum was exceeded by 204 per cent. Sham feeding during the course of 8 weeks caused a marked decrease. The daily work output in the final or third gelatin period was 895 per cent greater than in the final week of training, or 247 per cent greater than the peak of the second gelatin period.

In Group B the average individual gain in work output due to training was 8.5 per cent. The first gelatin period of 5 weeks' duration brought a 304 per cent increase in work. A slight decrease of 4 per cent was observed in the post-gelatin period. Sham feeding brought a further reduction. In the last or second gelatin period the work output was increased by 531 per cent over that of the last week of training, and was 74 per cent greater as compared to the peak of performance in the first gelatin period.

Kaczmarek concludes his paper as follows, "The beneficial results that came with training or judiciously prolonged exercise were insignificant by contrast with those affected by gelatin feeding. The greater gains in work output were correlated with a decreased frequency of heart beat and pulse rate. The limits to the influence of exercise came to the fore during sham feeding. It was in this period that a decreased work output accelerated the heart and subsequent pulse rate. It was found in the course of 30 weeks of riding that a definite amount of work (the training maximum) gave, in 10 of the 12 subjects, a higher heart beat and pulse rate reaction during sham feeding than either in the preceding or in the subsequent period. The other two subjects gave trends that deviated from this norm. Their heart and pulse rate response, however, did conform to type during this period when they rode to full fatigue. In these two subjects the lessened work output of sham feeding was correlated with higher heart and

pulse rates. The psychic influence induced with the onset of sham feeding was short-lived, and of little or no consequence in thwarting the descending trend in work output and in the ascending curves of both the heart beat and pulse rate."

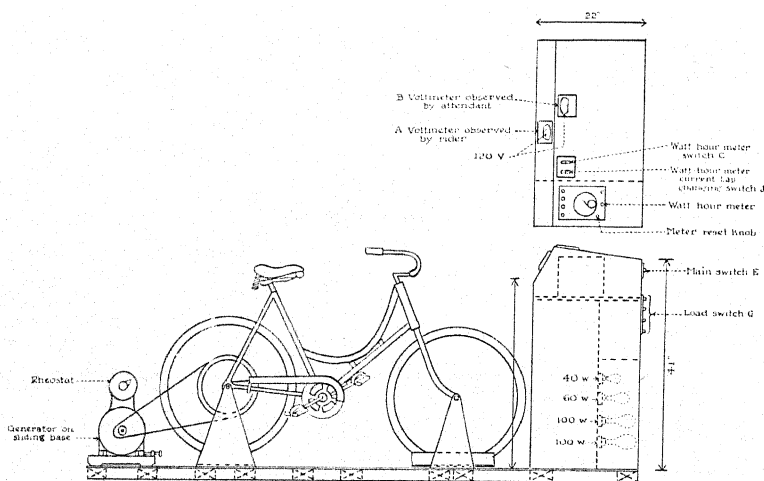
These extensive studies of Professor Kaczmarek offer conclusive evidence that in his subjects the feeding of gelatin does increase greatly the work output. The writer is of the opinion that the negative results of the other workers listed above have no bearing on the results of Ray and Kaczmarek. Definite conclusions, however, in this connection should be withheld until more evidence is available on the subject.

Since gelatin is made up of about 25 per cent of the amino acid glycine (together with large amounts of arginine), and since both these amino acids are precursors of muscle creatine, it is only natural to expect that glycine, or amino acetic acid, should be tested in increasing the work output in normal adults. Furthermore about 58 per cent of protein can be changed into carbohydrate in the body and this would also be used as a source of energy.

McGuire (1) discussed the effect of glycine in the treatment of asthenia in 1934. He stated, "It must be confessed that we know little about weakness, fatigue and exhaustion. We have no way to measure them, no pathology to explain them, and no way, except by rest, to cure them. A patient who without cause gets unduly weak and tired after only moderate exertion is certainly not normal. Would it not be well to admit the kinship of these conditions with the other recognized myopathies and dignify them collectively with the term 'myasthenia mitis' because of the mild resemblance of their symptoms to myasthenia gravis?"

He then goes on to state "Glycine is more of a food than a medicine. The benefit of glycine is analogous to a vacation. In neither can marked results be seen from day to day. The benefit of both is cumulative and is only appreciable after a

lapse of considerable time. The beneficial symptoms do not become manifest until the patient has been taking glycine for 3 to 6 weeks. The patient awakes one morning with an unaccustomed feeling of strength and vigor. Muscles that have felt tired and heavy now feel refreshed and lighter, and there is a desire to undertake tasks that before were irksome and



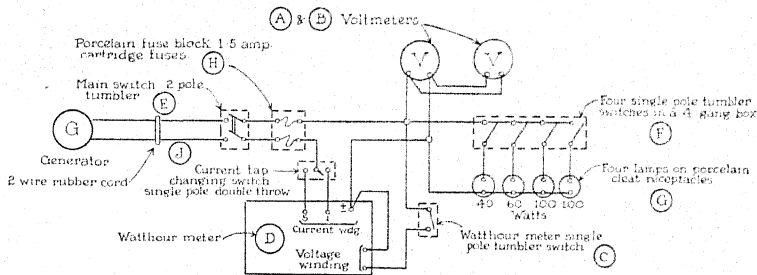
Ergometer

Fig. 1.

difficult. There is a new tone (probably due to an increased creatine formation) to all the voluntary muscles. The tired sagged expression of the face changes and the soft flabby muscles of the extremities become firm and hard. The patient gains weight and strength and while there is occasional periods when there is a return of the old lassitude and weakness, life for him is altogether different from what it was before."

Beard (35) showed in 9 underweight, but otherwise normal individuals who had been unable to gain weight by other means, that the daily ingestion of glycine caused an increase

in strength and body weight during one to 24 weeks. Glycine ingestion has been of much benefit in the following conditions: non-specific asthenia (Boothby (8, 9), Hensch (1)); anorexia (Beard (35)); easy fatigability (Wilder (1)); nervousness (Beard (35)); asthenia (Wohl and Pastor (1), Terhune and Green (1)). The case histories of the patients in these studies are very interesting and are no doubt responsible for the wide therapeutic popularity of glycine (amino



(Fig. 2. Wiring Diagram.)

acetic acid) at the present time, in cases of asthenia and also in the treatment of the myopathies. This aspect of the question will be discussed in Chapters XVI and XVII.

Beard (31, 36) tested out glycine, with and without urea, and several other substances upon human endurance, using the bicycle ergometer (Figs. 1 and 2). In the first study (31) the effect of ingesting glycine, with and without urea was studied. It will be recalled that the writer has shown that creatinuria and the concentration of muscle creatine in the rat were doubled when urea and glycine were injected as compared to the effect of either alone (11, 12). Creatinuria in man was likewise doubled when these substances were ingested together (glycine-urea, or sarcosine-urea) as com-

pared to their effect when ingested alone (12, 34). The results obtained in our first study with volunteers riding the bicycle ergometer (31) are given in Fig. 3.

Glycine in wine, glycine with urea in wine, and the wine base alone were ingested by young men and women in order

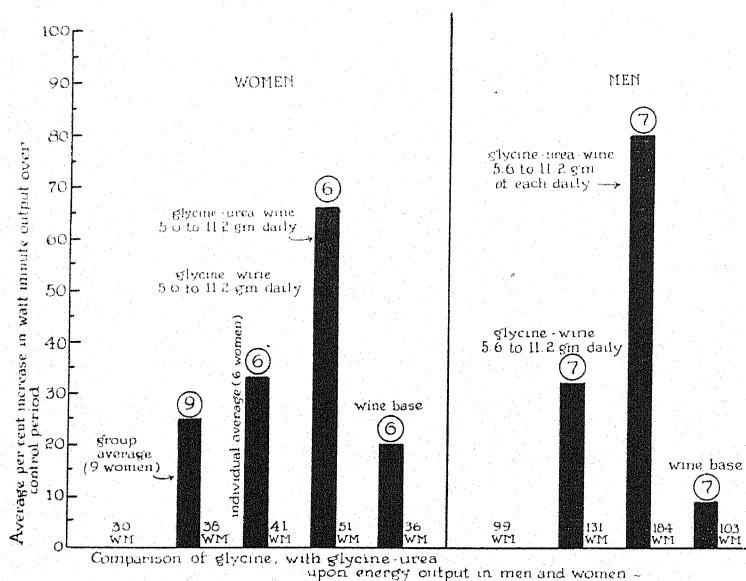


Fig. 3.

to test the effect of each of these products upon the duration of exercise (watt minute output) when they rode the bicycle ergometer at 120 volts against a load of 45 watts. Glycine in wine caused an increase of 22 per cent in watt minute output over the control value in the women as a group. Individually, however, these increases ranged from 16 to 62 per cent, or an average of about 32 per cent. Glycine with urea caused an average increase of 66 per cent in watt minute out-

put over the control value. Glycine caused in men, with two exceptions, an average increase of 32 per cent, and glycine with urea an increase of 79 per cent, over the control value. Ingestion of the wine base alone at the end of the study caused a rapid loss (within 3 days) of this increased power of endurance back again to the control level. These results were not due to a training effect or to the ingestion of the small amount of wine base alone. The results of this study show that each individual has a natural energy output which, however, can be increased as long as glycine, with or without urea, is ingested. It required 3 weeks for increases in energy output to manifest themselves.

In our second study (30) various other substances were employed. The 75 medical students performed a total of 2,755 tests as follows: control, 450; first experimental period, 825; second experimental period, 1,009; and wine base period, 471. The main object of the tests was to determine the ability of the student to ride the ergometer against a load of 105 watts, keeping the pointer on the voltmeter as close to 120 volts as possible during the tests (Figs. 1 and 2). The time in seconds that they could do this was recorded, and from this value the average watt minute output of each student was calculated.

The products ingested were as follows:

1. Glycine-urea dissolved in a wine base. In this product various other substances were also dissolved, or added, such as crystalline vitamins B₁, B₆, synthetic α -tocopherol, and calcium glycerophosphate. Creatine and creatinine, with and without the phosphate, were dissolved in the wine base alone. Glycine in wine, glycine tablets and glycine-urea tablets were also used.

The average results given in Fig. 4 and 5 show that the watt minute output was doubled when glycine, or glycine-

urea, either in the form of wine or tablets, was ingested at the 10 gm. daily level as compared to the 5 gm. daily level; likewise at the 5 gm. level as compared to the 2.5 gm. level.

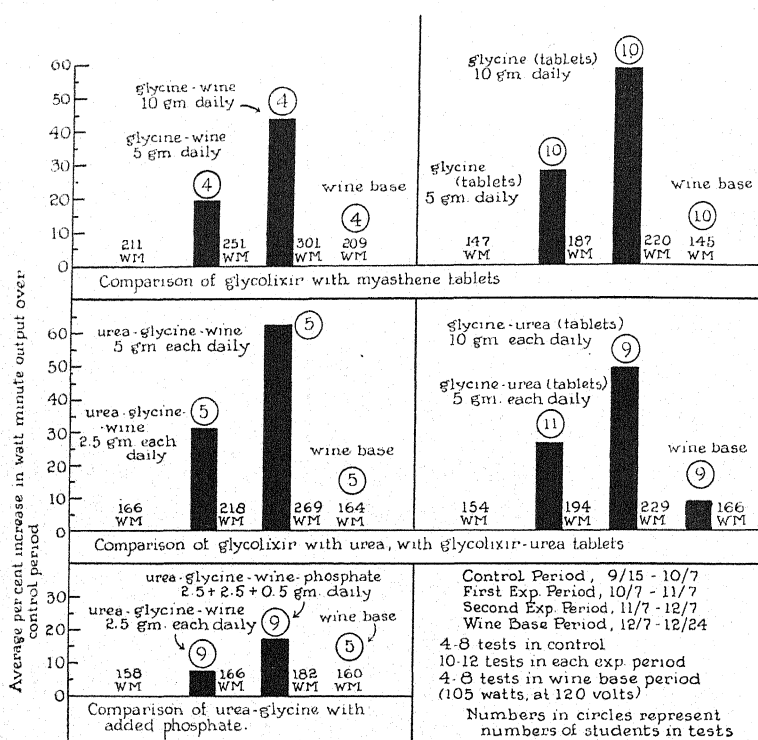


Fig. 4.

This finding confirms the results of our first study discussed above (31) and again shows that the watt minute output is directly proportional to the daily amount of these substances ingested. The addition of phosphate to the urea-glycine-wine product seemed to show a somewhat higher watt minute

output than was obtained from the same amount of urea-glycine alone. The results of this study were, however, unsatisfactory since five of these students did not show an increased watt minute output in any period and four addi-

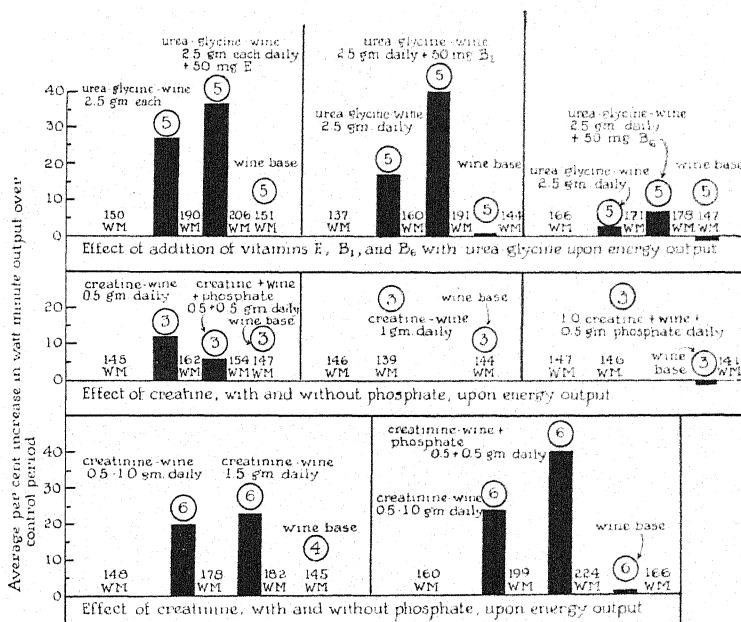


Fig. 5.

tional students withdrew from school during the tests. The former criticism can also be made of the studies where vitamins B₆ and E were used.

Evidence was obtained in five students that the daily ingestion of 50 mg. of vitamin B₁ with urea-glycine increased the watt minute output about 22 per cent above that obtained from the urea-glycine alone. Gounelle (1) showed that cyclists who had ingested 0.005 gm. of vitamin B₁ kept ahead

of the other cyclists in a bicycle race. A state of muscular euphoria and resistance to fatigue was also noticed in these subjects. Similar results were obtained in football players. McCormick (1) has observed improvement in certain physical tests when B_1 was administered in 14 subjects. Guerrant and Dutcher (1) showed that physical exercise increases the B_1 requirement of the rat. Briem (1) reported that the addition of vitamin B_1 to Ringer's solution distinctly delays fatigue in frog muscle and intensifies considerably the action of acetyl choline. The addition of 12 mg. per cent of phosphates to the solution reduced the amount of vitamin B_1 requirement from 1,000–2,000 to 10–1,000 γ per cent in the rectus abdominis muscle and from 200 to 400 to 1–20 γ per cent in the sartorius muscle. The amount of vitamin B_1 necessary to increase the action of acetyl choline is reduced from 1,000–2,000 to 10–100 γ per cent in the rectus abdominis muscle and from 20–60 to 10–40 γ per cent in the sartorius muscle. R. D. Williams, *et al.* (1) showed that a lack of vitamin B_1 in otherwise normal individuals would lead to fatigue. In two of these subjects increased work performance was observed after ingesting this vitamin. Since B_1 plays such an important part in carbohydrate metabolism it is to be expected that its administration would serve to increase physical fitness.

Much evidence from the writer's laboratory has shown that, contrary to the previously accepted view, creatine is not changed into creatinine in the body (15, 17, 18, 22, 23, 24, 26, 37). It was also shown that the body metabolizes administered creatine and creatinine in a different manner from the creatine and creatinine formed during the course of protein metabolism. It is also well known that the myopathy patient does not utilize administered creatine to any great extent.

In view of these findings creatine or creatinine were administered dissolved in the wine base alone. The ingestion of creatine, with or without added phosphate, did not increase the watt minute output very much above the control level. On the other hand the administration of creatinine did so and the addition of phosphate almost doubled the watt minute output as compared to that obtained from the ingestion of the same amount of creatinine alone. This observation is important for it demonstrates again that creatinine can be changed into creatine, which, as phosphocreatine, plays an important part in muscular contraction. It has also been observed by others that phosphate ingestion serves to increase the energy output (Kaunitz and Austria (1); Bøje (1); Atzler, *et al.* (1); Raibuschinsky (1); and Freeman (1)). Of interest in this connection is the finding of Emmelin and Kahlsan (1) who showed that creatinine increased the sensitivity of frogs' muscle to acetyl choline. Although creatinine itself has no effect it may occasionally produce contraction.

After December 7th the wine base alone was ingested by all students who had been taking the wine products and the ingestion of the tablets was discontinued in the other students taking these tablets. In all students, with one exception, the increased watt minute output dropped to the control level within three days after ingestion of the wine base was started. This is interesting in view of the fact that it usually requires about three weeks for increased endurance to be shown after ingestion of the above products. About eighty per cent of the tests were taken from 5 to 5:30 p.m. daily except Saturdays. At this time each day the student had been sitting or standing on his feet in classes since 9 a.m. They should, therefore, show their maximum mental and physical fatigue at the time that the tests were given.

Questionnaires were given the students at different times

during the conducting of the tests and the answers to these revealed the following facts: (a) There was considerable loss of sleep and worrying about grades by the whole class; (b) the worry continued, but more sleep was obtained by the class as a whole after ingesting the products; (c) there was an increase in appetite; (d) the increased endurance was real; (e) muscle pains experienced at the beginning of the tests soon disappeared; (f) increases of a few pounds in weight were observed in some students; (g) most of the students felt better than before the tests began; and (h) many students were much disappointed at losing their increased energy output after ingesting the wine base alone.

Of much interest was the fact that fifteen students did not show any increased watt minute output over their control level during the whole study. (Their average watt minute output in the four periods was 156, 157, 156, 158.) This shows that a one to two minute period of vigorous exercise twice weekly for three months did not serve to increase the watt minute output due to a training effect. This was also true of the rest of the students since their increased endurance was rapidly lost in the wine base period.

The following correlation coefficients were calculated:

<i>Variables</i>	<i>Correlation Coefficient</i>
Body weight—average watt minute output	$r = -0.54 \pm 0.06$
Age—average control watt minute output	$r = -0.06 \pm 0.08$
Average control watt minute output—average watt minute output during second month after ingesting different products	$r = -.004 \pm 0.08$

Chaikelis (1) made 14 different measurements (hand grip, chinning, push ups, lift tests, 60 and 220 yard dashes, blood pressure, pulse rates, etc.) totaling 1,632 observations on 19 control and 40 experimental subjects during a 10

week period. The controls received placebo tablets containing glucose and lactose and the experimental subjects received glycolixir (glycine) tablets. The results obtained are listed in Table 38.

Under the influence of glycine a number of these tests showed results that were definite improvements over the initial efforts. The grip strength improved by 22.5 per cent and 23.1 per cent for the right and left hand, respectively. The lifting strength improved 12 and 22.8 per cent, respectively, for the back and leg muscles. The total body strength measured as the modified Rogers' strength index showed an improvement of 17 per cent. The total creatinine excretion showed a drop of 29.2 per cent as compared to that of the initial excretion level. The increase of about 23 per cent in the strength of the leg muscles corresponds to our findings (Fig. 3 and 4) of about 25 to 35 per cent increase in watt minute output when the students rode the bicycle ergometer after glycine ingestion.

Foltz, *et al.* (1) used double work periods in their studies with caffeine. A bicycle ergometer with a rather heavy load was used, 1,235 KgM per minute with a pedalling rate of 54 RPM. Three times per week the subjects worked to exhaustion, and after 10 minutes of rest were worked to exhaustion again. Caffein (0.25 gm.), injected intravenously immediately after the first work period, increased the per cent recovery in 2 out of 4 subjects.

These results again prove, as stated above in regard to our results with glycine, that each individual *may or may not give increases in energy output or relief of fatigue under identical experimental conditions*. These individual physiological variations should always be taken into consideration in evaluating the action of any substance upon increasing the energy output in man.

Rôle of Glycine in Metabolism. Since this amino acid has come into wide use in the treatment of asthenia and diseases of the muscles and heart, its rôle in the metabolic processes of the body will be discussed here. The beneficial effects of

TABLE 38
(After Chaikelis (1))

COMPARATIVE ANALYSIS OF THE RESULTS OBTAINED ON A CONTROL SERIES (19 SUBJECTS) AND ON AN EXPERIMENTAL GLYCOCOLL SERIES (40 SUBJECTS), BOTH SERIES COMPOSED OF NORMAL, HEALTHY, ATHLETIC AND NON-ATHLETIC YOUNG MEN OF COLLEGE AGE, WITH REGARD TO EACH OF 14 DISTINCTIVE AND SEPARATE TESTS

ANALYSIS	BODY WEIGHT	BODY HEIGHT	PROTOPLASMIC MASS	PULSE RATE PER MINUTE	BLOOD PRESSURE		BARACH INDEX
					Sys-tolic	Dias-tolic	
	lbs.	inches	lbs.				
<i>Control series:</i>							
Mean (A) of initial observations ..	146.6	64.6	92.14	72.	110.	65.	126.
Mean (B) of terminal observations ..	148.4	64.8	93.47	71.	109.	67.	119.
Mean of the amount of change between initial and terminal observations ..	2.2	0.16	0.8	-1.9	1.	1.8	-1.6
ϵ^* of mean change ..	2.7	0.2	0.9	4.5	3.2	4.4	7.9
$\epsilon_M \dagger$ of the mean change ..	0.633	0.045	0.211	1.063	0.760	1.045	1.873
<i>Glycocoll series:</i>							
Mean (A ₁) of initial observations ..	149.4	67.8	99.11	72.	110.	66.	130.
Mean (B ₁) of terminal observations ..	152.7	67.9	100.20	71.	111.	68.	128.
Mean of the amount of change between initial and terminal observations ..	3.20	0.12	1.21	-1.95	0.25	1.00	-2.50
ϵ^* of mean change ..	3.7	0.1	1.1	7.7	5.0	4.5	16.3
$\epsilon_M \dagger$ of the mean change ..	0.859	0.034	0.247	1.720	1.126	0.997	3.654
Difference between means of change of Control and Experimental Series ..	1.00	0.04	0.41	0.05	0.75	0.80	0.93
‡ Standard error of the above difference ..	1.068	0.056	0.325	2.022	1.357	1.450	3.720
Critical ratio: Observed diff./Standard error ..	0.9	0.71	1.26	0.02	0.55	0.55	0.25
Chances of obtaining a difference equal to or greater than the observed difference due to errors of random sampling ..	46 in 100	24 in 100	10 in 100	49 in 100	48 in 100	48 in 100	40 in 100

$$* \epsilon = \text{mean deviation} = \sqrt{\frac{\sum d^2}{N-1}}$$

$$\dagger \epsilon_M = \text{mean deviation of the mean} = \frac{\epsilon}{\sqrt{N}}$$

$$\ddagger \text{Standard error of the difference between means} = \sqrt{(\epsilon_{M1})^2 + (\epsilon_{M2})^2}$$

the amino acid under these conditions should be considered as a whole, since the formation of creatine is only one of its functions.

Glycine can be used in the detoxication of many com-

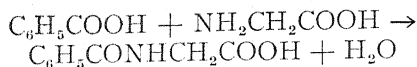
TABLE 38—Continued

(After Chaikelis (1))

COMPARATIVE ANALYSIS OF THE RESULTS OBTAINED ON A CONTROL SERIES (19 SUBJECTS) AND ON AN EXPERIMENTAL GLYCOCOLL SERIES (40 SUBJECTS), BOTH SERIES COMPOSED OF NORMAL, HEALTHY, ATHLETIC AND NON-ATHLETIC YOUNG MEN OF COLLEGE AGE, WITH REGARD TO EACH OF 14 DISTINCTIVE AND SEPARATE TESTS

GRIP-STRENGTH		LIFT-STRENGTH		PUSH-UPS (DIPS)	PULL-UPS (CHINS)	MC CLOY ARMS STRENGTH INDEX	RUNNING		ENDURANCE INDEX	TOTAL CREATI- NE CONC. IN MG. PER 100 CC.	MODIFIED ROGERS INDEX
Right hand	Left hand	Back mus- cles	Leg mus- cles				60 yd. dash	220 yd. dash			
lbs.	lbs.	lbs.	lbs.				secs.	secs.			
265.	241.	284.	367.	8.5	8.5	243.	8.5	32.5	3.84	226.	1400.
294.	248.	281.	354.	8.5	9.6	250.	8.5	32.9	3.86	243.	1427.
29.	17.	0.3	-18.	0.0	1.0	7.3	0.9	0.4	0.01	17.	26.8
34.9	53.6	29.6	50.1	1.2	2.2	11.6	0.8	0.6	0.1	35.2	69.9
8.216	12.622	6.966	11.805	0.272	0.512	2.737	0.199	0.138	0.027	8.300	16.482
253.	229.	267.	322.	7.0	7.0	241.	8.7	32.9	3.80	235.	1312.
310.	273.	299.	394.	8.0	9.0	256.	8.5	32.3	3.81	162.	1532.
57.	53.	32.30	73.40	0.88	1.30	12.	-0.18	-0.57	0.005	-68.50	223.50
37.5	27.6	44.3	63.1	2.0	1.7	9.5	0.3	1.1	0.1	44.9	119.1
8.382	6.186	9.916	14.113	0.449	0.373	2.129	0.056	0.246	0.030	10.054	26.640
28.00	36.00	32.00	91.40	0.88	0.30	4.70	1.08	-0.97	0.005	-85.50	196.70
11.740	14.060	12.120	18.400	0.270	0.630	2.860	0.210	0.280	0.030	13.040	29.700
2.38	2.56	2.64	4.97	3.22	0.47	1.64	5.22	3.44	0.16	6.56	6.62
87 in	52 in	41 in	0 in	6 in	32 in	5 in	0 in	3 in	44 in	0 in	0 in
10,000	10,000	10,000	10,000	10,000	100	160	10,000	10,000	100	10,000	10,000

pounds in the body. A classical example of this is the detoxication of benzoic to form hippuric acid, as follows:



Glycine arises chiefly from the metabolism of the proteins in the body. However, exogenous glycine may also be able to detoxify benzoic acid. A normal man can detoxify about 5 gm. of benzoic acid in 12 hours. This reaction occurs chiefly in the liver (Quick (1, 2)). Quick has also demonstrated that the synthesis of hippuric acid is directly related to liver function and that this synthesis is impaired in various diseases of the liver. Hence the ability of the liver to form hippuric acid has been used successively as a test for liver function by Quick (1), Teregulov (1) and Snapper and Grunbaum (1).

In the toxemias of pregnancy the ability to detoxicate benzoic acid is impaired (Neuweiler (1), Herscheimer (1)). This indicates that glycine ingestion may be of benefit in this disease. Bach (1) stated that glycine may conjugate with aldehydes and ketones and, therefore, it may play a rôle in the regulation of the oxidative metabolism in the body. It is very possible that further investigation will indicate that glycine ingestion may play an important rôle in the detoxication of disease toxins. Glycine ingestion up to about 40–50 gm. daily is nontoxic to man.

Glycine conjugates with cholic acid to form glycocholic acid, one of the bile acids, which plays an important rôle in the absorption of fats from the intestine. It is readily converted in the body into other amino acids, such as glutamic, aspartic, tyrosine, arginine, etc., and in turn is readily synthesized from other compounds (Ratner, *et al.* (12)). P. R. White (1) showed that glycine is an essential factor for the

growth of bacteriophage and that tomato roots grow well with glycine as the sole source of nitrogen.

The specific dynamic action of glycine is well known (Rapport and Beard (39, 40, 41); Wilhelmi and Mann (1, 2); Wilhelmi (3); Lusk (1)). The degree of stimulation varies with the amount ingested and with the nutritive state of the animal. The specific dynamic action of the amino acids is observed chiefly in the liver, but the metabolism of other organs may also be affected.

Glycine can be quantitatively transformed into glucose and glycogen in the body (Butts, *et al.* (1), MacKay, *et al.* (1)). The ammonia which is liberated in these reactions is converted into urea by the liver and this causes an increase in the urea content of the urine and blood. Prolonged feeding of glycine to anemic dogs stimulates the synthesis of hemoglobin (Whipple and Robscheit-Robbins (1)). It also accelerates the production of bile salts (Whipple and Smith (2)).

Glycine is combined with cysteine and glutamic acid to form the tripeptide, glutathione, a substance which probably plays an important part in metabolic reactions. This tripeptide is readily oxidized and reduced and is concerned with cellular respiratory reactions (Oppenheimer and Stern (1), Elvehjem and Wilson (1)). From the above considerations it is possible that glycine and glutathione are indispensable units in the metabolic reactions in the body.

Russo (1), Oehme (1) and Lacquet, *et al.* (1) have suggested the possible rôle that glycine plays in the functioning of the thyroid, pancreas and adrenals. Beard and Thiberge (42) employed glycine ingestion successfully in several allergic conditions when the usual methods of treatment had failed. In one patient, who before treatment was sensitive to meat, fish and eggs, there was a loss of this sensitivity to these foods and his extensive skin rash of 10

years' duration cleared up in a few months. Several other cases showed marked relief of their symptoms. Pastinszky (1) suggested the use of glycine in peptic ulcers.

The writer has information of the very beneficial results obtained in hundreds of cases of different diseases of the eye following ingestion of glycine (unpublished observations of a physician of New Orleans). This therapy deserves attention of ophthalmologists in the future.

Kalter (1) administered glycine to patients with decompensated heart disease and reported several cases in which it apparently exerted a beneficial effect and he was enthusiastic about the possibilities of glycine therapy. Similar results were reported by Herrmann and Dechard (2). In spite of the fact that clinical studies are hard to control it is certain that glycine therapy in heart disease should be given a thorough trial by clinicians in the future.

CHAPTER XVI

NUTRITIONAL MUSCULAR DYSTROPHY
AND CREATINE EXCRETION.
CREATINE-CREATININE
METABOLISM AND
THE VITAMINS

Nutritional Muscular Dystrophy and Creatine Excretion.
The etiology of various muscle diseases has long baffled clinicians. Many facts were obtained from a study of the normal and pathological histology of the muscles in these diseases. Physiological studies, chiefly with nerve-muscle preparations, have added to our information of the subject. Chemical analyses have lately been given prominence in this connection. The advances in the newer physiology of muscle function, (especially in enzyme action and the rôle the vitamins play as important parts of some of these enzymes) the significance of phosphate bond energy (in which creatine phosphate plays an important rôle), the relation of respiration to phosphorylation, the metabolism of glycogen and glucose, and the chemistry of muscular contraction,—all these, have recently focussed attention upon the muscles as the seat of many important biological reactions both in health and disease.

It was only natural then to expect that sooner or later the vitamins would be connected with diseases of the muscles

and nerves. Morgulis (3) has written an interesting review of nutritional muscular dystrophy.

Evans and Burr (1) in 1927 were the first to report that suckling rats born of mothers which had received just enough of vitamin E to permit successful reproduction, developed a characteristic paralysis. At the end of three weeks of life most of the young were paralyzed in the musculature and posterior extremities. The young grew well, but half of them soon died. Positive evidence that the deficiency was one of vitamin E was obtained by shifting the young of a normal rat to the vitamin-E-deficient mother. Paralysis and death of these young soon occurred. Feeding of natural food stuffs or those containing vitamin E prevented the occurrence of the paralysis.

Evans, *et al.* (2) observed a similar paralysis, the result of severe muscular dysfunction, in adult rats which had been fed a vitamin-E-deficient diet for about twenty-two months. The deficiency was characterized by degenerative lesions in the cross striated muscles, loss of longitudinal striations, multiplication of nuclei, hyaline degeneration and later calcification or replacement of muscle fibers by fat and connective tissue. Even before these changes a decrease in muscle power, in muscle creatine, and increased chloride concentration in the muscles, were associated with an increased excretion of creatine. MacKenzie, *et al.* (5) observed that tremors and incoordination of head and leg movements occurred.

Numerous workers have confirmed the findings of Evans and coworkers. A lack of vitamin E causes the nutritional muscular dystrophy and its administration cures the condition:

In the Rat. Goettsch (3); Morelle (1); Ringsted (1); Lipschutz (1); Butt, *et al.* (1); Olcott (1, 2); Barrie (1, 2); Einarson and Ringsted (2); Demole and Pfaltz (1); Pappenheimer (5); Goettsch and Ritzmann (4); Telford,

et al. (3, 4); Knowlton, *et al.* (1, 3, 4); Morris (1); Vezar (1); Pappenheimer and Goettsch (6); Aloisi and Polanyi (1); H. M. Evans, *et al.* (5, 6, 7); Krakower and Axtmayer (1); Holmes and Pigott (1).

In the Rabbit. Goettsch and Pappenheimer (7); Goettsch and Brown (8); Pappenheimer and Goettsch (9); Madsen, *et al.* (1, 2); Morgulis and Spencer (4); Morgulis (5); Morgulis, *et al.* (6); Cary (1); MacKenzie and McCollum (6, 7, 8, 9); MacKenzie (10); Mattill (1, 2); Morris (1).

(MacKenzie (11) produced as many as 6 successive attacks of nutritional muscular dystrophy in rabbits fed on vitamin-E-free diets with a similar number of cures by the administration of α -tocopherol. If this vitamin therapy was followed after the last attack complete repair of hyalinization and necrosis of the thigh muscles were observed.)

In the Chicken. Pappenheimer and Goettsch (10, 11, 12, 13); Pappenheimer and Graff (14); Pappenheimer, *et al.* (15); Goettsch and Pappenheimer (16, 17); Wolf and Pappenheimer (18); Jukes and Babcock (1); Elvehjem, *et al.* (2).

In the Mouse. Pappenheimer (19).

In the Hamster. Houchin (1).

In the Guinea Pig. Madsen (3); Wood and Hines (1); Shimotori, *et al.* (8, 9); Chor (1).

In the Dog. Anderson, *et al.* (3, 4, 5); Brinkhous and Warner (1).

In the Duck. Seifried and Heidegger (1).

Brinkhous and Warner (1) demonstrated the occurrence of nutritional muscular dystrophy, similar to that which occurs in vitamin E deficiency, in chronic biliary fistula dogs which were maintained on an adequate diet. The dystrophy was believed to be due to a vitamin E deficiency which results from a faulty absorption of this vitamin in the absence of bile from the intestine.

Rabbits and guinea pigs fed upon certain diets develop an extreme degree of muscular dystrophy of the skeletal muscles. The creatine content is lower than normal and is roughly proportional to the amount of pathological degeneration (Goettsch and Brown (2); Morgulis (3)). There is an increase in water and total nitrogen (Goettsch and Brown (2)), an increase in the rate of oxygen consumption (Victor (1); Madsen (3)), a decrease in glycogen, total acid soluble phosphorus, and fractions thereof, an increase in cholesterol (Morgulis (3)), and in sodium chloride, a corresponding loss in potassium and magnesium, and an increase in calcium (Fenn and Goettsch (1), Morgulis and Osherhoff (1)).

The increase in water content and retention of salt in these dystrophic muscles may well account for some of the creatinuria observed in these animals. The remainder of the creatinuria would originate from creatine liberated in the diseased muscle tissue and from a non-utilization of newly formed creatine in the muscles.

Goettsch, *et al.* (18) analyzed 50 normal and 50 dystrophic rabbits' muscle for total phospholipid, total acid soluble phosphorus, and total inorganic orthophosphate phosphorus. These values were then compared to the extent of the pathological degeneration and creatine content.

Their results show that no striking changes in the phosphate fractions occur until the muscles are severely degenerated. When calcification occurs there is an increase in total acid soluble and total inorganic orthophosphate phosphorus; in those without calcified fibers there is a decrease in these constituents. There is no change in the phospholipid phosphorus. The creatine phosphate content falls, but its relation to the total acid soluble phosphorus remains the same.

Lu, *et al.* (8) observed in paralyzed rats fed a vitamin-E-

deficient diet that there was a slight decrease in inorganic phosphate and in creatine phosphate, and a marked decrease in total acid soluble phosphorus and pyro and organic ester phosphorus, the last mentioned decrease accounting for about 65 per cent in the total acid soluble phosphorus. In spite of a decrease in muscle creatine the ratio of inorganic and creatine phosphorus to the total acid soluble phosphorus was not changed. In the paralyzed cases not markedly dystrophic no change occurred in the calcium content. In the case of two markedly dystrophic suckling rats different changes were found in the musculature. The limb muscles were white and waxy in appearance and microscopically were profoundly degenerated. There was a 50 to 180 per cent increase in calcium and a great increase in the total acid soluble phosphorus. These changes were accompanied by a greater decrease in creatine phosphate phosphorus. The ability to phosphorylate glycogen was decreased about 40 per cent in the paralyzed muscles and about 80 per cent in the markedly dystrophic group. *In vitro* additions of vitamin E did not restore this esterification of glycogen.

In the cases of nutritional muscular dystrophy referred to above there is a marked loss of muscle creatine accompanied by creatinuria. This creatinuria is prevented by ingestion of vitamin E. These changes occur in the rat (Telford, *et al.* (4); Vezar (1); Knowlton, *et al.* (4)); in the rabbit (Goettsch and Brown (8); Morgulis (3); MacKenzie and McCollum (1)); in children (Hottinger (1, 2)). Madsen (3) observed a lowered muscle creatine content in muscles of the guinea pig during nutritional muscular dystrophy in this species.

Almquist and Mecchi (2) showed that a dietary deficiency of creatine or its precursors results in nutritional muscular dystrophy in the chick. Jukes (2) showed that, in contrast to turkeys, chicks fed on a simplified diet deficient in choline

did not develop perosis unless gelatin or creatine was added. Choline prevented the perosis at a level of 0.1 per cent. Jukes suggested that the tension exerted on the bones by the muscles plays a part in causing the distortion of the bones which characterizes perosis. If the tension is reduced by muscular dystrophy as in creatine deficiency, the tendency towards perosis may be lessened.

Lipschutz (1) studied the histological changes which occur in these dystrophies. There are two types of paralysis, a weak and a spastic type. One part of the homolateral descending vestibular tracts is destroyed first, then the crossed tracts also become diseased as the deficiency of vitamin E increases, which leads to the spastic type of paralysis. Einarson and Ringsted (2) also made an extensive study of the nervous and muscular systems of rats suffering from vitamin E deficiency. These investigators have demonstrated degeneration of the pyramidal tract and anterior horn cells in their animals and have called attention to an analogy with the lesions observed in amyotrophic lateral sclerosis in man. They also discuss points of similarity and divergence between the symptoms of vitamin B complex deficiencies and vitamin E deficiencies in animals. They believe that the components of the vitamin B complex might also require the coexistence of vitamin E for their activity.

Cathcart, *et al.* (3) were the first to determine the creatine content of denervated muscle in cats and rabbits. After the 15th day there is a decrease in muscle creatine. Working on rats Hines and Knowlton (6) showed a loss of creatine on the third day which is the time of onset of fibrillation in this species. At the end of the first week the creatine content of the muscles was much lowered as compared to the controls. At the end of 28 days the creatine phosphate had diminished to 30 per cent of the control value, while the adenosine-

triphosphate and the undetermined phosphate fraction were diminished to 50 per cent in a similar time. The loss of these two fractions was proportional to the loss in muscle tissue. The same findings have been reported by Avellone and di Macco (1).

Tower (1), in her review of the subject, has summarized the changes that occur in muscle after denervation as follows: 1. General protoplasmic constituents, no change. 2. Substances related to the muscle fiber and reduced in proportion to its atrophy. 3. Substances found chiefly in the interstitial tissues and increasing with relative and absolute increase in these tissues. 4. Substances especially related to interstitial tissues, but increasing disproportionately. 5. Substances found especially in muscle tissue, but disproportionately reduced in conjunction with fibrillation.

In the first category are total nitrogen (proteins) and water. (In their study of the atrophy of denervated muscle, however, Chen, *et al.* (1) found after the end of 10 weeks that 50 per cent of the muscle protein was lost.) In the second category are potassium, acid soluble phosphorus, creatine, adenosine triphosphate and perhaps phospholipid. In the third category are chloride and inorganic phosphate. In the fourth category is calcium. In the fifth category are glycogen and creatine phosphate. Lactic acid and phosphoric acid constitute a sixth category of substances produced in fibrillatory activity.

Chor (1) has described the histological changes produced by feeding to guinea pigs the diets used by Goettsch and Pappenheimer and Madsen, *et al.* (1, 2, 3). The pathologic changes were those of a coagulative necrosis. Despite marked degeneration of muscle fibers the intramuscular nerves and their terminals are well preserved. This type of muscle lesion was, therefore, considered to be extraneural in origin. The

histologic alterations characteristic of this experimental myopathy are different from those observed in nutritional muscular dystrophy in man and the term "nutritional myodegeneration" rather than "dystrophy" was suggested by Chor for these lesions.

Thomson, *et al.* (7) observed that, following denervation in the rat, there was a progressive decrease in muscle creatine, mass and strength as elicited through maximum direct stimulation and total absence of response to motor nerve stimulation. This continued until functional reinnervation occurred in 2 or 3 weeks. Thereafter atrophy was arrested and a progressive recovery of muscle creatine, mass and strength was noted. At the end of 12 weeks the regenerating muscles showed 85 to 90 per cent of normal mass and strength. The creatine concentration values closely paralleled the changes in the muscle cell phase.

Pappenheimer (19) studied muscular dystrophy in mice fed on vitamin-E-deficient diets. Female mice were maintained on an E-low diet and one dose of E was given to insure the normal birth of the young. In 20 per cent of 293 mice necrosis of the fibers was observed. Mice killed the first day showed edema of the subcutaneous and intramuscular tissues. In about one third of the cases hyaline necrosis of the muscle fibers were observed. In a group of mice sacrificed at 16 to 35 days of age muscular lesions were observed in 59 per cent of the animals. There was early calcification of necrotic fibers and active regeneration. Adult mice occasionally show scattered hyaline or calcified fibers remaining from the early lesions, but no progressive dystrophy of the muscles. No lesions of significance were found in the central nervous system or in other tissues and organs. In mice the spermatogenesis was active on the vitamin-E-free diets up to 439 days.

H. M. Hines and Knowlton (5) observed that the administration of 200 mg. of glycine per day was ineffective upon the rate of denervation atrophy in the rat. Richter (1) has confirmed this finding with larger doses of glycine over a longer period of time. These results are to be expected since regeneration of muscle tissue requires *all* of the necessary substances in the diet, including proteins, fats, carbohydrates, salts, water, and vitamins. Holmes and Pigott (1) showed that in young weanling rats which had developed various stages of muscular dystrophy due to a vitamin-E-deficient diet there was a definite response to the oral administration of massive doses (4 to 14 I.U.) of thiamin hydrochloride per rat.

From the above results it is possible that some of the myopathies which occur in man might be simple deficiency diseases of the neuromuscular system. While the majority of evidence points to a lack of vitamin E in this connection it should be remembered that vitamin deficiencies as they occur in man are usually multiple rather than single deficiencies and the ingestion of other vitamins than E have been suggested in these conditions. This aspect of the question will be discussed in Chapter XVII.

Creatine-Creatinine Metabolism and the Vitamins. The relation between the vitamins and creatine-creatinine metabolism has recently been studied in the writers' laboratory (Beard and Pizzolato (43, 44)). Young rats of 100-150 gm. body weight were used and were fed our usual stock diet. Different amounts of the crystalline vitamin preparations were dissolved, or dispersed in 1 cc. of physiological saline, and injected parenterally into the rats. In the first study the effect of this treatment upon the concentration of muscle creatine was observed. The results obtained are listed in Table 39.

TABLE 39

EFFECT OF PARENTERAL INJECTION OF CRYSTALLINE
VITAMINS UPON THE CONCENTRATION OF MUSCLE
CREATINE IN THE RAT
(Beard and Pizzolato (43))

Vitamin	Amount In- jected, Time, Rats, mg. Days No.			(MUSCLE CREATINE)			Increase Over Controls, %
				Max., %	Min., %	Av., %	
Control			36	0.45	0.39	0.42	12.0
B ₁ HCl	10	1	3	0.48	0.46	0.47	12.0
B ₁ HCl	20	2	3	0.44	0.40	0.42	None**
B ₁ HCl	30	1	3	0.45	0.41	0.43	None
B ₁ HCl	40	1	3	0.48	0.44	0.45	None
B ₁ HCl	20*	3	5	0.47	0.40	0.43	None
B ₂	10	1	3	0.48	0.46	0.47	12.0
B ₂	20	2	3	0.47	0.40	0.44	None
B ₂	30	1	3	0.47	0.40	0.43	None
B ₂	40	1	3	0.48	0.44	0.45	None
B ₂	20*	3	5	0.50	0.47	0.49	16.6
B ₁ Pyrophosphate	10	1	3	0.45	0.39	0.42	None
B ₁ Pyrophosphate	20	1	3	0.44	0.41	0.43	None
B ₁ Pyrophosphate	20*	3	5	0.46	0.35	0.41	None
B ₁ Pyrophosphate	60	3	5	0.46	0.39	0.42	None
B ₆ HCl	10	1	3	0.50	0.44	0.46	9.5
B ₆ HCl	20	2	3	0.43	0.36	0.40	None
B ₆ HCl	30	1	3	0.51	0.47	0.49	16.6
B ₆ HCl	40	1	3	0.52	0.50	0.51	21.4
B ₆ HCl	20*	3	5	0.62	0.53	0.57	35.7
B ₆ HCl	30	1	6	0.62	0.50	0.56	33.3
Nicotinic Acid	10	1	3	0.52	0.49	0.50	19.1
Nicotinic Acid	20	2	3	0.42	0.40	0.41	None
Nicotinic Acid	20	1	3	0.57	0.49	0.53	26.2
Nicotinic Acid	20*	3	5	0.46	0.40	0.43	None
Nicotinamide	10	1	5	0.43	0.41	0.42	None
Nicotinamide	20	1	3	0.44	0.42	0.43	None
Nicotinamide	30	1	3	0.45	0.41	0.43	None
Nicotinamide	40	1	3	0.41	0.41	0.41	None
Vit. C Ascorbic Acid	10	1	3	0.52	0.49	0.50	26.2
Vit. C Ascorbic Acid	20	2	3	0.42	0.38	0.40	None
Vit. C Ascorbic Acid	20*	3	5	0.40	0.39	0.39	None
Vitamin E	10	1	3	0.50	0.46	0.49	16.6
Vitamin E	20	2	3	0.44	0.41	0.42	None
Vitamin E	20*	3	5	0.45	0.42	0.43	None
Vitamin E	50	1	3	0.44	0.39	0.42	None
Vitamin E	100	1	2	0.54	0.54	0.54	28.6
Vitamin E***	100	2	5	0.55	0.48	0.51	21.4
Methyl Naphthoquinone	10	1	3	0.48	0.46	0.47	12.0
Methyl Naphthoquinone	20	2	3	0.46	0.43	0.44	None
Methyl Naphthoquinone	20*	3	5	0.44	0.39	0.41	None

* 20 mg. daily for 3 days. All of the other doses were injected at one time, i.e., on the first day of the experiment. At the end of 1 to 3 days later the animals were killed and the muscle creatine determined.

** Less than 10%, or insignificant.

*** 200 mg. vitamin E gave identical results obtained in these studies as 100 mg.

From these results it is seen that vitamin B₁, vitamin B₂, pyrophosphate (cocarboxylase), nicotinamide, and 2 methyl naphthoquinone gave little or no increase in the creatine content of the muscles. Vitamin C (ascorbic acid) gave an increase of 26 per cent, which, however, may have been due to the fact that this vitamin itself gives the Jaffé reaction with alkaline picrate. Vitamin B₆ (pyridoxine HCl), nicotinic acid, and vitamin E (α -tocopherol) gave increases in muscle creatine. Both nicotinic acid and pyridoxine may aid in the production of those respiratory enzymes containing the pyridine structure which is involved in muscle metabolism. It was concluded that these two vitamins together with vitamin E must be considered as essential for the normal functioning of muscle tissue in the body.

Beard and Pizzolato (44) injected various vitamins into adult rats fed upon their stock diet and the effect of this treatment upon the excretion of creatine and creatinine in the urine above the control values for these substances was determined. Vitamin A (ester produced by molecular distillation), thiamin chloride, pyridoxine HCl, riboflavin, nicotinic acid, viosterol, biotin, 2 methyl naphthoquinone and calcium pantothenate did not influence the excretion of either creatine or creatinine. The injection of cocarboxylase and cebione (Vitamin C) caused a retention of creatinine. Vitamin K₁ caused a retention of creatinine in 3 studies and an increased excretion of creatinine in another. Vitamin E (α -tocopherol) caused an average retention of 156 mg. of creatinine in 4 studies which was followed by an increased excretion of creatine, as creatinine, of 146 mg. It is, therefore, possible that this vitamin influences the transformation of creatinine into creatine in the tissues (Table 40).

Plehwe (1) and Fischer and Oehme (1) showed that the creatinuria produced by the injection of thyroxin can be abolished by addition of vitamin C. Plehwe also showed that

TABLE 40
EFFECT OF PARENTERAL INJECTION OF CRYSTALLINE VITAMINS UPON CREATINE-
CREATININE EXCRETION
(After Beard and Pizzolato (44))

Exp. No.	Amount of Vitamins Injected	Experi- mental Period, Days	CREATININE			CREATINE (AS CREATININE)			Creatine mg.	Ex- creted, mg.	Con- trol, mg.	Re- tained, mg.	Ex- creted, mg.	Vitamin A concentrate (200,000 USP units per gm.)
			Expt., mg.	Re- tained, mg.	Ex- creted, mg.	Con- trol, mg.	Re- tained, mg.	Ex- creted, mg.						
1	2,600 units	4	114	6		120			24	4				
2	10 mg.	4	166		14	152			40	1				
3	33 mg.	4	264	8		272			72				4	
4	33 mg.	3	181	26		207			25	9				
5	33 mg.	3	161	46		207			21	7				
6	10 mg.	4	123	15		138			26	2				
7	33 mg.	3	193	17		210			25	10				
8	33 mg.	3	196	29		225			32	15				
9	10 mg.	4	127	1		128			14				15	
10	33 mg.	3	129	18		147			14					
11	33 mg.	4	102	24		126			21				1	
12	33 mg.	4	239			224		15	24	3				
13	33 mg.	3	223			147		76	39	16			25	
14	33 mg.	4	188	20		168			31					
15	33 mg.	4	257	7		264			80				1	
16	1,000 units	4	232			232			77				16	
17	33 mg.	4	218	26		244			56				21	
18	33 mg.	4	227	40		187			41				56	
19	33 mg.	3	18	10		102			83				43	
20	33 mg.	6	92	10		102			15				22	
21	100 units	6	188	24		268			27				25	
22	100 units	5	145	7		152			17				16	
23	33 mg.	4	130	14		144			25					
24	33 mg.	4	223	21		244			60	1				
25	33 mg.	4	95	13		108			20				17	
26	33 mg.	6	159	66		225			18				2	
27	33 mg.	3	257			162		95	32	6			5	
28	33 mg.	3	207	1		192		15	35				5	
			188			189			50	23				

* Vitamin A (natural ester, General Mills, Inc., Minneapolis) and Viosterol (A. R. P. I. process, in oil, Distillation Products Co., Rochester, N. Y.).

** Merck & Co., Rahway, N. J.

*** Highly purified concentrate of biotin (vitamin H) prepared from liver by Dr. Paul György, University Hospitals, Cleveland, Ohio.

the creatinuria of Basedow's disease could be suppressed by this vitamin. Hirata and Suzuki (2) showed that vitamin C in doses of 200–500 mg. daily over a period of 6 months, decreased the creatine-creatinine excretion, lowered the blood phosphorus to normal, and increased the glycogen and creatine phosphate in the muscles. These workers (1) also showed that in progressive muscular dystrophy there is a lack of vitamin C in the cerebrospinal fluid and the excretion of this vitamin is only about 25 per cent of that of normal individuals. The intravenous administration of large doses of vitamin C increased the creatine and phosphorus with a notable improvement in the condition of the patients. The excretion of creatine and creatinine were reduced and their normal ratio was again established. Fan and Woo (2) showed that, in a case of glycogen storage disease, saturation with vitamin C caused a marked increase in the excretion of creatine and creatinine. Palladin and Epelbaum (4) reported a decrease in muscle creatine in severe scurvy. Handovsky (1) stated that 100 mg. of creatine per kg. stimulates the formation of vitamin C (ascorbic acid) in the pigeon. Ratsimamanga (1) showed that the creatine phosphate content of the adrenals of guinea pigs fed on a meat diet was reduced to one third their normal value by this vitamin.

If it were not for the fact that vitamin C itself reduces alkaline picrate to alkaline picramate (which gives a red color which cannot be distinguished from that produced by creatinine in alkaline picrate) the observations listed above between vitamin C and creatine-creatinine metabolism would be of considerable interest. In future studies in this connection the absence of vitamin C from muscle filtrates and urine must be shown. If the free vitamin should be present in the tissue or fluid it will then be necessary to use the specific creatinine enzyme of Miller and Dubos to positively identify

the color of the Jaffé reaction as being due to creatinine and not to vitamin C.

Vasile and Pecorella (1) showed that the administration of vitamins B₁ and B₂ caused the physiological creatinuria to disappear in normal infants. The total creatinine excretion also decreased after administration of either vitamin. The results were stated to be due to the pronounced glycogen-fixing action of these vitamins.

CHAPTER XVII

METABOLISM IN THE HUMAN MYOPATHIES. EFFECT OF INGESTION OF AMINO ACIDS AND VITAMINS UPON CLINICAL RESULTS AND CREATINE-CREATININE METABOLISM

Creatine-Creatinine Metabolism in the Myopathies. The myopathies comprise many different types of disease. These have recently been classified by Airing and Cobb (1). Major Rizzio stated that 25,000 men out of 720,000 were rejected for army duty from the United States, due to musculo-skeletal defects. It would seem, therefore, that these diseases are far more common than was formerly believed. The etiology of these diseases is not well understood. Within recent years, however, due to the discovery of the effect of amino acids upon the formation of muscle creatine and to the fact that nutritional muscular dystrophy can be produced by faulty diets in different species of animals, new light has been focused on these diseases of the muscles. More progress has been made in the last 10 years in treating the myopathies than ever before. The creatine-creatinine metabolism, and recently the metabolism of phosphorus, have been most thoroughly studied. Men may excrete some creatine (Beard (32)), and this is also true of women and children. This is considered physiological creatinuria. In the studies on creatinuria in the myopathies the water intake of the patient

should not be more than 2,000 cc. per day since the ingestion of large quantities of water will influence the creatine output (Beard, *et al.* (22)). It is also a mistake to feed the patient on a diet low in protein. A normal protein diet will not usually influence the creatine-creatinine excretion and is necessary to meet the protein needs of the body. *If a normal protein diet is not fed the administration of glycine will not form muscle creatine and the patient will have no benefit from its ingestion.* Under these conditions the administered glycine, gelatin, or other protein will contribute first to the needs of protein. The formation of muscle creatine is a secondary reaction and occurs only after the protein requirements are satisfied.

Few analyses of the muscle tissue in the human myopathies have been made. Nevin (1, 2); Reinhold and Kingsley (2); Hirata and Suzuki (2); Collazo, *et al.* (1); Debre, *et al.* (1) all observed that there was a decrease in creatine, creatine phosphate, adenosine triphosphate and other phosphate fractions in cases of muscular dystrophy. The analogy here with the chemical analysis of muscle tissue in animals suffering from nutritional muscular dystrophy is striking, in that both the animal and human diseases are characterized by a lowering of the creatine and phosphate compounds and an increase in water. Fenn and Goettsch (1) showed that there was also an increase in sodium and chloride and a decrease in potassium and phosphate. These findings are of much interest in view of the theory of the writer that some of the creatine excreted in the urine of the myopathy patient may be formed from creatinine by the addition of water (*Cf.* Chapter X). Fibrillation occurs most commonly after nerve section which then causes degeneration. Tower (1) showed in denervation atrophy that there was a loss in glycogen, potassium, and creatine phosphate.

The excretion of both creatine and creatinine has been

most extensively studied in the myopathies. In some of these diseases there is little change in the excretion of these substances, in others there is a creatinuria which may be accompanied by creatinine retention (Table 41).

TABLE 41

CREATINE-CREATININE EXCRETION IN THE MYOPATHIES

<i>Myopathy</i>	<i>No. of Cases</i>	<i>Creatine gm./24 hours</i>	<i>Creatinine gm./24 hours</i>	<i>Observer</i>
Myasthenia Gravis	1	0.30	0.90	Milhorat and Wolff (10)
Myasthenia Gravis	1	0.47	0.70	Cooke and Passmore (1)
Myasthenia Gravis	30	0.04	1.23	Adams, <i>et al.</i> (2)
Prog. Musc. Dystrophy	5	0.39-0.65	0.14-0.63	Harris (8)
Prog. Musc. Dystrophy	1	0.24	0.80	Milhorat and Wolff (11)
Prog. Musc. Dystrophy	1	0.39	0.38	Milhorat and Wolff (11)
Myotonia Congenita	1	0.05	1.82	Milhorat and Wolff (9)
Myotonia Congenita	1	0.02	1.88	Milhorat and Wolff (9)
Myotonia Atrophica	1	1.39	0.96	Morgulis and Young (7)
Amyotonia Congenita	1	0.06	0.08	Milhorat and Wolff (9)
Amyotonia Congenita	1	0.98	0.97	Ziegler and Pearce (1)
Amyotonia Congenita	1	0.11	0.06	Powis and Raper (1)
Amyotonia Congenita	1	0.15	0.07	Milhorat and Wolff (9)

Brand and Harris (7), Friedburg and West (1), Linneweh and Linneweh (1), and Thomsen (4) have shown that the muscular dystrophy patient could synthesize hippuric acid as easily as normal subjects, thereby synthesizing glycine in liberal amounts. But the analogy here probably does not hold. Even though the patient can synthesize liberal amounts of glycine under these conditions, it should be remembered that this is a detoxifying or emergency reaction, while the synthesis of glycine (or its utilization in metabolic reactions)

TABLE 42

EFFECT OF DIFFERENT TYPES OF THERAPY UPON CREATINE EXCRETION AND CLINICAL RESULTS IN THE MYOPATHIES

<i>Myopathy</i>	<i>No. of Cases</i>	<i>Creatinuria</i>	<i>Therapy</i>	<i>Clinical Results</i>	<i>Observer</i>
Myasthenia Gravis	87	++	Glycine	Excellent improvement in 50 per cent of the cases	Boothby (10)
Myasthenia Gravis	6		Prostigmin	Much improvement	Winkelman and Moore (1)
Myasthenia Gravis	3	+	Glycine	Good improvement	Reese, <i>et al.</i> (1)
Myasthenia Gravis	4		Prostigmin	Excellent improvement	Lindsey (1)
Myasthenia Gravis	1	++	Glycine and Prostigmin	Prostigmin effective. No effect with glycine	Cooke and Passmore (1)
Myasthenia Gravis	3		Glycine and Prostigmin	Best results when used together	Riven and Mason (1)
Myasthenia Gravis	2		Guanidine-HCl	Improvement	Minot and Frank (1)
Myasthenia Gravis	5		Guanidine-HCl	Improvement	Minot, <i>et al.</i> (2)
Myasthenia Gravis	26		Guanidine-HCl	Improvement	Dodd, <i>et al.</i> (3)
Myasthenia Gravis	2		Glycine and Ephedrine	Striking improvement	Schmitt, E. O. G. (1)

Myasthenia Gravis	2	Prostigmin	Continued improvement	Winkelman and Moore (1)
Myasthenia Gravis	2	Prostigmin	Improvement	Smith (1)
Myasthenia Gravis	5	Ephedrin	Symptoms relieved	Smith (1)
Myasthenia Gravis	4	Glycine	Symptoms relieved	Smith (1)
Myasthenia Gravis	2	Prostigmin, glycine, and ephedrin	Good results on all three	McFarlane (1)
Myasthenia Gravis		Prostigmin and atrophine	Best results in 30 minutes	Friez and Marno (1)
Myasthenia Gravis	2	Prostigmin and Physostigmin	Excellent results	Milhorat (13)
Myasthenia Gravis		Prostigmin	Excellent results in 30 minutes	Schoor (1)
Myasthenia Gravis		Acetyl choline, mecholyl	Increased recovery of muscle power	Fraser, <i>et al.</i> (1)
Myasthenia Gravis	44	Prostigmin, ephedrin, KCl, or Guanidine	7 showed complete remissions; 5 died	Viets and Schwab (1)
Myasthenia Gravis	1	Glycine	Good response	Urechia and Retezeanu (1)
Myasthenia Gravis	2	Antitritin and Prostigmin	Much improvement	Meredith (1)

TABLE 42 (Continued)

<i>Myopathy</i>	<i>No. of Cases</i>	<i>Creatinuria</i>	<i>Therapy</i>	<i>Clinical Results</i>	<i>Observer</i>
Prog. Musc. Dystrophy	3	++	Glycine	Much improvement	Milhorat, <i>et al.</i> (4)
Prog. Musc. Dystrophy	14	++	Glycine	Much improvement in 2 cases	Milhorat, <i>et al.</i> (2)
Prog. Musc. Dystrophy	7	++	Glycine	Good improvement	Kostakow and Slauck (1)
Prog. Musc. Dystrophy	2	++	Glycine	Some improvement	Espersen and Thomsen (2)
Prog. Musc. Dystrophy	4	++	Glycine	Improvement in one case	Borst and Mobius (1)
Prog. Musc. Dystrophy	16		Glycine	Improvement in 7 cases	Kostakow (3)
Prog. Musc. Dystrophy	4	+	Glycine	Good improvement	Reese, <i>et al.</i> (1)
Prog. Musc. Dystrophy	22	++	Parathyroid and adrenal cortex	Marked clinical improvement in 15 cases	Berman (1)
Prog. Musc. Dystrophy	2	++	Glycine	Favorable results	Mader, <i>et al.</i> (1)
Prog. Musc. Dystrophy	9	++	Glycine	Improvement in structure and composition of muscles	Reinhold, <i>et al.</i> (1, 2)
Prog. Musc. Dystrophy	6		Vitamin B ₆	Considerable improvement in all cases	Antopol and Schotland (1)
Prog. Musc. Dystrophy	7		Vitamins E and B ₆	No improvement in any case	Ferrebee, <i>et al.</i> (1)

Prog. Musc. Dystrophy	8	Vitamin E + peanut oil	No improvement in 5 months	Shelden, <i>et al.</i> (1)
Prog. Musc. Dystrophy	27	Glycine	Decrease in fatigability and improvement in nutritional condition	Boothby (8)
Prog. Musc. Dystrophy	3	Vitamin D	Good results	Cornwell (1)
Prog. Musc. Dystrophy	3	Vitamin D	No results	Cornwell (1)
Prog. Musc. Dystrophy	2	Vitamin E	Marked clinical improvement in 1 case	Alpers, <i>et al.</i> (1)
Prog. Musc. Dystrophy	1	Glycine	Much improvement in the clinical condition	von Pastinsky (1)
Muscular Dystrophy	1	+	Good improvement	Reese, <i>et al.</i> (1)
Muscular Dystrophy	1	+	Objective and subjective im- provement	Roy, <i>et al.</i> (1)
Muscular Dystrophy	4	Glycine	Good improvement	Boshes (1) ●
Muscular Dystrophy	5	Gelatin	Objective improvement	Stone and Abeles (1)
Muscular Dystrophy	6	Prostigmin	Much improvement	Winkelman and Moore (1)
Muscular Dystrophy	2	Glycine	General improvement	Cuthbertson and MacLachlan (1)

TABLE 42 (Continued)

<i>Myopathy</i>	<i>No. of Cases</i>	<i>Creatinuria</i>	<i>Therapy</i>	<i>Clinical Results</i>	<i>Observer</i>
Muscular Dystrophy	1		Vitamin E	Brilliant recovery	Meller (1)
Muscular Dystrophy	4		Vitamin E	Marked improvement in 1 case	Alpers, <i>et al.</i> (1)
Muscular Dystrophy	1		Vitamin E	Much improvement	Donovan (1)
Muscular Dystrophy	18		Whole wheat germ	Definite improvement in 9 cases	Bicknell (1)
Muscular Dystrophy			Glycine	No improvement	Bech (1, 2)
Muscular Dystrophy			Glycine	No improvement	Schoor and Boer (1)
Muscular Dystrophy			Glycine	No improvement	Richter (1)
Muscular Dystrophy			Glycine	No improvement	Espersen and Thomsen (1)
Muscular Dystrophy			Glycine	No improvement	Dieckhoff (1)
Muscular Dystrophy			Glycine	No improvement	Cooke and Passmore (1)
Muscular Dystrophy			Glycine	No improvement	Faure-Breaulieu and Wohl (1)
Muscular Dystrophy	1		Glycine	Good response	Urechia and Retezeanu (1)

Muscular Dystrophy	5	Vitamin E, yeast and gelatin	Definite improvement	Stone (1)
Muscular Dystrophy	1	Prostignin	Temporary improvement	Winkelman and Moore (1)
Muscular Dystrophy	5	Glycine	Slight improvement in 1 case	Braestrup (1)
Muscular Dystrophy		Vitamin E	Beneficial results	Stone (1)
Muscular Atrophy	2	++ Glycine	General improvement	Cuthbertson and MacLachlan (1)
Muscular Atrophy	1	Vitamin E, yeast and gelatin	Definite improvement	Stone (1)
26 Prog. Musc. Atrophy	1	Gelatin	Objective improvement	Stone and Abeles (1)
Prog. Musc. Atrophy	14	Vitamin E	3 recovered, 7 improved	Meller (1)
Prog. Musc. Atrophy		Glycine	Good results	Razgha, <i>et al.</i> (1)
Prog. Musc. Atrophy		Vitamin C	Objective and subjective improvement	Hirata and Suzuki (1)
Prog. Musc. Atrophy	2	Vitamin E	No improvement	Doyle and Merritt (1)
Prog. Musc. Atrophy	2	Glycine	No improvement	Bargi (1)
Ps. Musc. Atrophy	3	++ Glycine	Much improvement	Milhorat, <i>et al.</i> (4)

TABLE 42 (Continued)

<i>No. of Cases</i>	<i>Creatinuria</i>	<i>Therapy</i>	<i>Clinical Results</i>	<i>Observer</i>
Myopathy Ps. Musc.				
2	++	Glycine	Good improvement	Mettel and Slocum (1)
Ps. Musc. Dystrophy				
5	++	Glycine	General improvement	Cuthbertson and MacLachlan (1)
Ps. Musc. Dystrophy				
16	+++*	Pilocarpin and adrenalin	Some improvement	Hough (1)
Ps. Musc. Dystrophy				
14	++	Glycine	No results in late cases	Mettel (2)
Ps. Musc. Dystrophy				
2	+++	Glycine	Progress of disease was arrested	Bender (1)
Prog. Ps. Musc. Dystrophy				
3	+++	Gelatin	Improved muscle function	Kisner, <i>et al.</i> (1)
Prog. Ps. Musc. Dystrophy				
6		Epinephrin and Pilocarpin	Temporary improvement	Kite (1)
Ps. Musc. Dystrophy				
8		Theelin, Mg and Choline	Striking improvement in all cases	Branch (1)
Ps. Musc. Dystrophy				
13		Vitamin E	No improvement	Ferrebee, <i>et al.</i> (1)
Ps. Musc. Dystrophy				
1		Vitamin E	No improvement	Doyle and Merritt (1)



Ps. Musc.	2	Vitamins E and B ₁	Excellent results	Stone (2)
Dystrophy				
Ps. Musc.	2	Glycine	Subjective improvement	Lowe (1)
Dystrophy				
Ps. Musc.	2	Vitamin E	No improvement	Lowe (1)
Dystrophy				
Amyo. Lat.	1	Prostigmin	Much improvement	Winkelman and Moore (1)
Sclerosis				
Amyo. Lat.	1	Gelatin	Objective improvement	Stone and Abeles (1)
Sclerosis				
Amyo. Lat.	4	Whole wheat germ	2 died, 1 improved, 1 completely cured	Bicknell (1)
Sclerosis				
Amyo. Lat.	1	Prostigmin	Increase in fibrillary tremors	Winkelman and Moore (1)
Sclerosis				
Amyo. Lat.	6	Vitamin E + peanut oil	No improvement in 5 months	Sheldon, <i>et al.</i> (1)
Sclerosis				
Amyo. Lat.	2	Vitamin E	Complete recovery in 1 case; marked improvement in the other case	Wechsler (1)
Sclerosis				
Amyo. Lat.	20	Vitamin E	2 recovered; 4 markedly improved; 5 moderately improved; 2 had disease arrested; 2 died; 2 grew worse	Wechsler (2)
Sclerosis				
Amyo. Lat.	6	Vitamins E and B ₁	No improvement	Ferrebee (1)
Sclerosis				

TABLE 42 (Continued)

<i>Myopathy</i>	<i>No. of Cases</i>	<i>Creatinuria</i>	<i>Therapy</i>	<i>Clinical Results</i>	<i>Observer</i>
Amyo. Lat.	11		Vitamin E	No improvement	Denker and Scheinmann (1)
Sclerosis	7		Vitamin E	No improvement	Doyle and Merritt (1)
Amyo. Lat.	1		Gelatin	Objective improvement	Stone and Abeles (1)
Sclerosis	7		Glycine	Favorable influence on the regenerative stage of the disease	Gros (1)
Polionyelitis					
Polionyelitis	1		Vitamin E	Beneficial results	Stone (1)
Polionyelitis	2	++	Glycine and Prostigmin	Marked improvement	Wohl and Pastor (1)
Adiposis	2		Vitamin E	No improvement	Bicknell (1)
Tabes Dorsalis	1		Vitamin E	Improvement	Bicknell (1)
Amyotonia	2		Vitamin E	No improvement	Doyle and Merritt (1)
Congenita	1		Vitamin E	No clinical improvement	Fleischmann (1)
Congenita	2		Vitamin E	Excellent results	Stone (2)
Amyotonia	1		Vitamin B complex	No effect	Bicknell (1)
Congenita	2		Vitamin B complex	Dramatic relief of muscular weakness in 1/2 hour	Spies, <i>et al.</i> (1)
Peroneal	1		Vitamin B complex	No effect	Bicknell (1)
Muscular	4		Vitamin B ₆	Dramatic relief of muscular weakness in 1/2 hour	Spies, <i>et al.</i> (1)
Atrophy					
Pellagra					

Neuro-Muscular Atrophy	9	Vitamin E	6 improved, 3 did not improve	de Gutierrez-Mahony (1)
Primary Fibrositis	30	Vitamin E	All patients were completely relieved of all symptoms	Steinberg (1)
Secondary Fibrositis	20	Vitamin E	Improvement in 8 cases	Steinberg (1)
Dystrophia Myotonica	13	Glycine, ephedrin, pilocarpin	Beneficial results	Waring, <i>et al.</i> (1)
Parkinson's Disease	3	Vitamin B ₆	Improvement within 1 hour, which was maintained if therapy was continued	Rudesill and Weigand (1)

under normal conditions of metabolism is not a detoxication reaction at all. Furthermore the addition of glycine to the diet of any one, normal or otherwise, is of much benefit, especially in increasing the energy output when riding a stationary bicycle.

Further incidences of the excretion of creatine in diseases of the muscles will be found in Table 42.

To summarize:

In Myasthenia Gravis creatinuria is seldom seen. Creatinine excretion may be low.

In Myotonia Congenita there is usually no creatinuria.

In Myotonia Atrophica creatinuria is present.

In Progressive Muscular Dystrophy creatinuria is present accompanied by some retention of creatinine.

In Progressive Muscular Atrophy creatinuria is present possibly with some creatinine retention.

In Amyotonia Congenita creatinuria is present with abnormally high creatinine retention.

In Amyotrophic Lateral Sclerosis creatinuria is present possibly with some creatinine retention.

In Pseudohypertrophic Muscular Dystrophy creatinuria and creatinine retention are present.

In Paramyoclonus Multiplex a slight creatinuria is present.

In many cases an increased creatine excretion is followed by a corresponding degree of creatinine retention (Tripoli and Beard (8), Milhorat and Wolff (11)). According to Milhorat and Wolff (11) muscle function decreases parallel to the decrease in creatinine excretion and increase in creatine excretion. This may mean either of two things, that the

creatine lost from the muscles escapes transformation into creatinine and is then excreted into the urine, or that some of the creatine formed from creatinine or amino acids is not utilized in the muscles and is excreted.

Creatine can probably be formed in normal amounts in the myopathy patient, but due to the fact that there is damaged muscle tissue present this tissue cannot *utilize* some of the creatine formed and this creatine is then excreted into the urine. This is shown by the fact that the myopathy patient does not utilize exogenous creatine to any great extent, but may utilize creatine formed in the usual course of protein metabolism. The writer has also shown (Chapters IX and XIII) that the body metabolizes administered creatine and creatinine in a different manner from that formed in the usual course of protein metabolism. Creatine utilization also depends upon the amount of phosphate donors present in the tissues.

Gammon, *et al.* (1) have reviewed the recent literature on the human myopathies. They state: "The study of diseases of the voluntary muscles during the last 10 years has revived new impetus from several different sources. Investigations of the nature of creatinuria in muscle dystrophy and of the influence of glycine upon it formed one phase which was followed by direct investigation of the specific muscle compounds. The discovery of a disease in animals of dietary origin which resembles the dystrophy of human cases, and which can be prevented and cured by vitamin E, is a recent observation which, at the present time, is influencing studies on the human disease. Another disease of natural occurrence in goats, which resembles the congenital myotonia in man, has facilitated the study of the myotonic phenomenon. The observations of Walker (1) that eserine and prostigmin would relieve the weakness of myasthenia gravis coming at the time of development by Loewi (1) and Dale (1) of the

neuro-humoral hypothesis of the transmission of the nerve impulse, stimulated work upon the relation of acetyl choline, choline, and choline esterase to these diseases. From this work also developed the studies on the effect of potassium in various muscular conditions, especially its effects in myasthenia and its dramatic effects in periodic paralysis. As a result of these investigations new drugs of great efficacy have been introduced in the treatment of some of these myopathies."

In myasthenia gravis there is no change in the muscle constituents except for an increase in soluble ester phosphorus (Nevin (1), Reinhold and Kingsley (2) and Collazo, *et al.* (1)). Williams and Dyke, however, found the muscle creatine to be low in this disease. Adams, *et al.* (2) observed that the concentration of blood calcium, magnesium, sodium, potassium, phosphorus, glucose, urea, creatinine, amino acids and uric acid, was unchanged. Hicks and Mackay (1) observed that the serum esterase was elevated in myasthenia gravis. All of the results of experimental data point to the similarity of myasthenia gravis to a mild curarization and it would seem that the defect in this disease would be due to an elevation of the threshold of the motor end plates to the effects of nerve impulses reaching them. Several clinical studies have also shown that there is a relation between the thymus and myasthenia gravis.

Kennedy and Wolf (1) and Nevin (3) showed that quinine and procaine, respectively, increased the muscular weakness by their curare-like action. On the other hand drugs which antagonize curare, such as potassium ions, (Wilson and Wright (1)); guanidine (Feng (1); Minot, *et al.* (1, 2); Dodd, *et al.* (3)); eserine and prostigmin (Briscoe (1); Walker (1); Laurent and Walther (1)) have a beneficial action in myasthenia gravis. According to Milhorat (13) when gross changes in the metabolism of creatine

and creatinine occur in myasthenia gravis the prognosis is grave.

The effect of prostigmin was supposed to destroy the excess choline esterase and inhibit the destruction of this enzyme at the neuromuscular junction. But Steadman and Russell (1), McGeorge (1), Milhorat (13), and Pilcher (1) have found no increase in choline esterase in the serum and no destruction of choline esterase. Regardless of its action, however, it is certain that administration of prostigmin gives dramatic relief in myasthenia gravis, and if the patient does not give a response to this drug myasthenia gravis is eliminated in the diagnosis.

In myotonia the muscle is abnormally sensitive to potassium ions. The injection of potassium sets up a long-lasting contraction. In myotonia of goats (Harvey (1)) and of humans (Ravin (1)), epinephrin, which reduces the sensitivity of striated muscle to the stimulating action of potassium, reduces the myotonia. According to Gammon, *et al.* (1) desoxycorticosterone acetate, which lowers serum potassium, causes the disappearance of myotonia in the goat.

In familial periodic paralysis the serum and urine potassium are low and the administration of this salt will rapidly cure the attack (Aitken, *et al.* (1); Gammon (2); Gammon, *et al.* (3); Pudenz, *et al.* (1); and Ferrebee, *et al.* (2)). J. H. Talbott (1) has reviewed the literature of this disease. The fall in serum potassium is, however, not the complete cause of the attacks since Allott and McArdle (2) have shown that the serum potassium when lowered by epinephrin, sugar, and insulin does not cause the muscle weakness. The fall of potassium is, therefore, secondary to some more fundamental change. Since the urine potassium also falls it is evident that the potassium which leaves the muscles goes elsewhere in the body. Hence the weakness may be due to an abnormally high ratio of cellular potassium to that outside,

and the cure of the weakness may follow a reduction of that ratio due to the administration of the salt.

Brand and Harris (3) observed a lowering of creatine and acid soluble phosphates in this type of paralysis. Creatinuria has been reported in several cases (Pudenz, *et al.* (1), Ferrebee, *et al.* (2) and Mitchell (1)). Allott and McArdle (1) observed a low serum phosphate. Ferrebee, *et al.* (1) and Pudenz, *et al.* (1), however, found no change in serum potassium.

Kullmann, *et al.* (3) stated that overdosage with desoxycorticosterone acetate produced a condition similar to familial paralysis. Serum potassium dropped markedly and a flaccid paralysis developed. Administration of potassium salts raised the serum potassium level to normal and relieved the paralysis. According to Gammon, *et al.* (1) the disturbance in this type of paralysis is probably due to an endocrine imbalance and the disorder may be due to the effect of adrenalin and insulin on the muscle tissue.

Effect of Ingesting Amino Acids and Vitamins upon Clinical Results and upon Creatine-Creatinine Excretion. Soon after the discovery of the origin of muscle creatine from the amino acid glycine, it was to be expected that clinicians would begin to study the effect of ingesting amino acids in the treatment of these various myopathies. As was to be expected, differences of opinion have been expressed by clinicians of the therapeutic value of glycine in these conditions. While it may be presumptuous on the part of the writer to offer suggestions of a clinical nature, nevertheless many negative reports of the effect of glycine are not very convincing from a critical point of view. Let us enumerate some of these here.

In the first place the etiology of many of these myopathies is not well understood. There may be errors in diagnosis of some of the conditions. In the second place some of these

diseases involve the neuromuscular apparatus where improvement is not to be expected. In the third place not enough cases have been studied up to the present time to warrant the drawing of definite conclusions of any type of therapy. In the fourth place some of these diseases may be present in the patient for over 20 years. If glycine does not give any results in a few months both the patient and physician may become discouraged and the treatment is stopped. (For instance, Denker and Scheinman (1) administered only 100 mg. of vitamin E for 30 to 80 days to 11 patients suffering from amyotrophic lateral sclerosis, with negative therapeutic results.) In the fifth place the degree of possible recovery in a patient depends primarily on the amount of functional muscular tissue remaining in the patient and to the degree of degeneration present. If the fibrotic stage has been reached no therapy will be of any value. In the sixth place the amino acids have not been given for a sufficient length of time to determine the degree of recovery possible. In the seventh place very few follow up studies have been made.

Milhorat, *et al.* (4) were the first to publish the beneficial effects of glycine administration in the myopathies. Since this time this question has been studied by many clinicians. The results obtained with different types of therapy are listed above in Table 42.

Tripoli and Beard (8) studied the initial and subsequent creatine-creatinine excretion in 51 cases of myopathy representing 12 different types of these diseases. Thirty-eight of them were studied over a period of four years.

Table 43 gives the rate of creatine excretion before and after glycine administration in 14 patients who showed objective and subjective improvement over a period of two years under this regime. Of the 12 different clinical conditions studied the only ones showing clinical improvement were those of progressive muscular dystrophy, pseudohyper-

TABLE 43

CASES IN WHICH OBJECTIVE IMPROVEMENT WAS OBSERVED
(After Tripoli and Beard (8))

Case No.	Age	Amino Acid, 10-20 gm.	URINE CREATINE PERIOD (5 DAYS)				<i>Diagnosis and Remarks</i>
			Control, gm.	1st, gm.	2nd, gm.	3rd, gm.	
1-N.B.S.	37	Glutamic	0.44	0.73	0.52	0.42	Prog. musc. dystrophy
4-J.B.	34	Glutamic	0.92	1.48	2.11	1.14	Prog. musc. dystrophy
6-G.W.	12	Glycine	0.43	0.96	1.42	0.87	Prog. musc. dystrophy
7-S.W.	10	Glutamic	0.24	0.22	0.10	0.33	Pseudo musc. dystrophy
2-L.T.	8	Glutamic	0.29	0.91	0.51	0.51	Pseudo musc. dystrophy
3-W.P.R.	10	Glycine	0.28	0.55	0.30	0.29	Prog. musc. dystrophy
5-H.H.	12	Glutamic	0.31	1.02	1.00	1.02	Prog. pseudo musc. dys.
13-C.C.	4	Glycine	0.32	0.57	0.22	0.03	Pseudo musc. dystrophy
11-L.B.	43	Glycine	0.17	0.23	0.32	0.15	Myasth. (intraocular paresis, diplopia)
14-E.J.S.	55	Glycine	0.46	0.39	0.39	0.57	Myasthenia of eyelids
8-R.M.B.	11	Glutamic	0.23	0.42	0.19	0.30	Early spino musc. atrop. Progress ar- rested
9-C.E.C.	28	Glycine	1.63	0.96	0.51	0.63	Strabismus with ciliary weakness
10-J.H.C.	54	Glutamic	0.12	0.36	0.76	0.19	Myasth. right eyelid with diplopia
12-A.S.	31	Glutamic	0.22	0.85	0.00	0.18	Paramyoclonus multiplex
		Average	0.43	0.69	0.60	0.47	
		Per cent increase		60.0	39.5	9.3	

trophic muscular dystrophy, paramyoclonus multiplex, myasthenia gravis with and without strabismus, and diplopia. In Table 44 will be found the follow-up studies of the same 14 patients 3 or 4 years later. It is seen that only 5 have maintained their improvement during this time since many of them discontinued the treatment. The results shown in this table indicate that glycine must be continually ingested if clinical improvement is to be maintained.

Our creatine-creatinine studies are listed in Table 45 (Beard, *et al.* (7)). For purposes of comparison we may divide the cases into three groups, as follows:

Group 1 (cases 1-10). Patients in whom the average creatine excretion rose from 50 to 200 per cent above that of the control period (provided this increased creatinuria soon disappeared in the third or later periods); showed both objective and subjective improvement.

Group 2 (cases 11-17). Some neuromuscular patients, who showed about the same degree of increase and decrease in creatinuria as those of Group 1; showed only subjective improvement or had arrest of their symptoms.

Group 3 (cases 18-30). Patients in whom no increased creatinuria occurred or where it was less than 50 per cent above that of the control period before amino acid therapy was instituted; showed no improvement (Table 46).

As a result of these studies it was suggested that a study of the extent of the creatinuria before and after glycine administration was a better diagnostic and prognostic test in the myopathies than was the creatine tolerance test.

As a result of all of the studies listed in this chapter it is evident that administration of glycine, or amino acetic acid, serves to increase the amount of creatine which is excreted in the urine of many of these patients. This lasts for about 2 weeks in which the amount of creatine formed from glycine is too great to be completely utilized and stored in the

TABLE 45

CREATINE-CREATININE METABOLISM IN VARIOUS MYOPATHIES AFTER AMINO-ACID THERAPY.
(AVERAGE 24-HOUR EXCRETION IN DIFFERENT PERIODS.) (AVERAGE VALUES FOR EACH GROUP.)
(Beard, Andes and Tripoli (7))

Patient No.	Age, Years	—CONTROL PERIOD—			—FIRST PERIOD—			—SECOND PERIOD—			—THIRD PERIOD—		
		Total Creatinine, gm.	formed Creatinine, gm.	Prec. Creatinine, gm.	Total Creatinine, gm.	formed Creatinine, gm.	Prec. Creatinine, gm.	Total Creatinine, gm.	formed Creatinine, gm.	Prec. Creatinine, gm.	Total Creatinine, gm.	formed Creatinine, gm.	Prec. Creatinine, gm.
Group 1 1-10*	8-37	1.17	0.73	0.50	1.38	0.72	0.77	1.16	0.56	0.10	1.14	0.65	0.57
		Amino Acid, 10-15 gm. Daily			Glycine acid or glycine			Glycine acid or glycine			Glycine acid or glycine		
		% Increase			% Increase			% Increase			% Increase		
Group 2 11-17†	12-54	1.15	0.82	0.38	1.72	1.08	0.74	1.38	0.99	0.45	0.97	0.81	0.18
		Glutamic acid or glycine			Glutamic acid or glycine			Glutamic acid or glycine			Glutamic acid or glycine		
		% Increase			% Increase			% Increase			% Increase		
Group 3 18-30‡	7-55	1.49	0.94	0.64	49.5	32.9	94.7	20.0	20.7	15.8	None	None	None
		Glutamic acid or glycine			Glutamic acid or glycine			Glutamic acid or glycine			Glutamic acid or glycine		
		% Increase			% Increase			% Increase			% Increase		
		10.0			13.8			None			None		

Results.—Group 1, objective and subjective improvement; Group 2, subjective improvement; Group 3, no improvement.
* Cases 1 to 10 were: Pseudohypertrophic muscular dystrophy, 4; Progressive muscular dystrophy, 3; and 1 each of "Psychopathic inheritance complex" (disuse atrophy), early spinomuscular atrophy and strabismus.

† Cases 11 to 17 were: Amyotrophic lateral sclerosis, 4; and 1 each of hypothyroid-hypopituitary, multiple sclerosis and spinomuscular dystrophy.

‡ Cases 18 to 30 were: Pseudohypertrophic muscular dystrophy, 3; progressive spinomuscular atrophy, 2; poliomyelitis, 2; and 1 each of "Friedreich's ataxia," tabes dorsalis with muscular dystrophy, muscular atrophy following radial nerve injury, von Recklinghausen's disease, "Jamaica ginger paralysis" and paresis with muscular atrophy.

FOLLOW-UP (3-4 YEARS) OF CASES SHOWING INITIAL OBJECTIVE IMPROVEMENT
(After Tripoli and Beard (8))

TABLE 44

<i>Case No.</i>	<i>Age</i>	<i>Diagnosis</i>	<i>Remarks</i>
1-N.B.S.	37	Prog. musc. dystrophy	Untraced
4-J.B.	34	Prog. musc. dystrophy	No improvement; therapy stopped
6-G.W.	12	Prog. musc. dystrophy	Improvement temporary; stopped therapy. Bedridden
7-S.W.	10	Prog. musc. dystrophy	Therapy Oct., 1933, to Sept., 1934. Remission
15-B.G.	10	Prog. musc. dystrophy	Improvement temporary. Stopped therapy. Bedridden
2-L.T.	8	Pseudo musc. dystrophy	No change. Stopped therapy. Bedridden
3-W.P.R.	10	Pseudo musc. dystrophy	Improvement temporary. Stopped therapy. Bedridden
5-H.H.	12	Pseudo musc. dystrophy	Improvement maintained on therapy. Almost normal
13-C.C.	4	Pseudo musc. dystrophy	Improvement maintained on therapy. Almost normal
11-L.B.	43	Myasthenia gravis	Improvement temporary. Stopped therapy. Same
14-E.J.S.	55	Myasthenia gravis	Improvement maintained two years. Untraced
8-R.M.B.	11	Early spino musc. atrophy	Improvement temporary. Stopped therapy. Same
9-C.E.C.	28	Ciliary muscle weakness	Improvement for 1½ years, then death. Cerebral hemorrhage
10-J.H.C.	54	Myasthenia and diplopia	Improvement maintained on therapy to date
12-A.S.	31	Paranyoclonus multiplex	

TABLE 46

CASES IN WHICH NO OBJECTIVE IMPROVEMENT (OR SUBJECTIVE IMPROVEMENT ONLY) OCCURRED
(Tripoli and Beard (8))

Case No.	Age	Amino Acid, 10-20 gm.	URINE CREATINE PERIOD (5 DAYS)				<i>Diagnosis and Remarks</i>
			Control, gm.	1st, gm.	2nd, gm.	3rd, gm.	
1	27	Glutamic	0.73	1.67	1.09	0.38	Amyotrophic lateral sclerosis
2	22	Glutamic	0.64	0.93	0.17	0.18	Amyotrophic lateral sclerosis
3	39	Glutamic	0.10	0.09	0.18	0.08	Amyotrophic lateral sclerosis
4	54	Glutamic	0.07	0.07	0.07	0.10	Amyotrophic lateral sclerosis
31	54	Glutamic	0.47	0.39	0.88	0.92	Amyotrophic lateral sclerosis
5	12	Glutamic	0.68	1.63	1.08	0.22	Hypothyroid-pituitary
36	10	Glycine	0.33	0.26	0.74	0.60	Progressive muscular dystrophy
35	8	Glycine	1.14	1.33	1.27	1.23	Progressive muscular dystrophy
9	50	Glutamic	1.19	1.64	0.91	1.39	Pseudo muscular dystrophy
17	12	Glutamic	0.42	0.34	0.30	0.29	Pseudo muscular dystrophy
30	5	Glycine	0.07	0.11	0.10	0.07	Pseudo muscular dystrophy
14	16	Glutamic	1.28	0.68	0.76	0.78	Pseudo muscular dystrophy
6	33	Glutamic	0.09	0.03	0.07	0.16	Multiple sclerosis
18	25	Glycine	0.00	0.12	0.00	0.00	Poliomyelitis
20	7	Glycine	0.15	0.14	0.14	0.12	Poliomyelitis
26	7	Glycine	0.27	0.34	0.21	0.24	Poliomyelitis
27	10	Glycine	0.13	0.32	0.41	0.36	Poliomyelitis

TABLE 46 (Continued)

Case No.	Age	Amino Acid, 10-20 gm.	URINE CREATINE PERIOD (5 DAYS)			Diagnosis and Remarks
			Control, gm.	1st, gm.	2nd, gm.	3rd, gm.
28	7	Glycine	0.20	0.29	0.31	0.30
34	24	Glycine	0.78	0.99	0.81	1.23
13	21	Glutamic	1.26	0.80	0.74	1.20
33	18	Glycine	0.37	0.58	0.15	0.14
25	8	Glutamic	0.03	0.44	0.24	0.42
8	52	Glutamic	0.40	0.94	1.47	1.31
11	11	Glycine	0.38	0.33	0.44	0.32
7	28	Glycine	0.37	1.49	0.96	0.00
32	5	Glycine	0.34	0.35	0.35	0.45
19	24	Glycine	0.37	0.27	0.18	0.20
21	7	Glycine	0.17	0.37	0.27	0.71
22	7	Glycine	0.74	0.68	0.53	0.31
23	6	Glycine	0.56	0.46	0.28	0.23
24	3	Glycine	0.37	0.23	0.36	0.91
29	24	Glutamic	0.17	0.31	0.25	0.29
10	36	Glutamic	1.43	0.28	0.42	0.69
16	38	Glutamic	0.36	0.36	0.28	0.25
15	21	Glutamic	0.33	0.48	0.53	0.40
12	55	Glutamic	0.93	1.23	0.16	0.06
		Average	0.48	0.58	0.47	0.35
		Per cent increase		21.0	None	None

muscles. During the third week and thereafter the creatinuria returns to its original level in spite of continued glycine administration. Utilization of this retained creatine is then shown by improvement in the clinical condition of many of the patients.

It must not be supposed that muscle structure and function depend solely on creatine and creatinine metabolism. The amino acids serve many other useful functions in the body such as their synthesis into body proteins, their specific dynamic action, some form sugar and most of them form creatine, some of them are interchangeable in metabolism, readily shifting their nitrogen from one to another. In some cases of pseudohypertrophic muscular dystrophy after amino acid therapy the calf muscles of the leg may lose their fatty and fibroaerolar tissue and protein is synthesized to take their place. It would seem, therefore, that the best therapy for the myopathies in the future would consist of the administration of a diet rich in protein with glycine, phosphates, and vitamins in addition to suitable exercises.

A few brief comments upon some of our cases will be mentioned here. A case of infantile paralysis of 2 years' duration in a young woman was much improved by the ingestion of glutamic acid (Tripoli and Beard (5-8)). Improvement in muscle function was observed around the lesion in the right leg and her general strength, which was decreasing at the time of glutamic acid ingestion, was greatly improved. This is of much interest since Schaffer (1) has shown that patients suffering from poliomyelitis may later develop muscular dystrophy. If amino acid therapy could be started in children as soon as this condition is diagnosed it is likely that much benefit would be obtained.

A case of extraocular paresis with vertical diplopia and ptosis of the eye lids was studied. After several weeks of glycine therapy the condition was much improved and at the

end of a year all symptoms of the disease had disappeared. It was no longer necessary for the patient to wear a shaded lens over his affected eye while lecturing. His improvement has persisted for the last 6 years in which time he has been symptom free.

Beard and Thiberge (42) treated several cases of severe allergic conditions with glycine. A 12 year old boy had asthma and eczema of the arms and legs since the second year of life. He was underweight and sensitive to meat, fish, eggs and bananas. Eight months after beginning glycine therapy he had gained 30 pounds in weight, due in part to an increased appetite. The skin on his arms and legs was dry and the rash had completely disappeared. At this time he could also eat anything. It was considered that the amino acid therapy provided a check, but not a cure, of his rather hopeless condition. Two other patients in this series obtained very beneficial results from this type of therapy and 4 others showed some improvement. It is of much interest in this connection that L. W. Hill (1) stated that 19 out of 36 cases of infantile eczema responded well to feeding with a synthetic food in which 60 per cent of the nitrogen was in the form of amino acids.

From these results it is possible that amino acid ingestion may be of distinct advantage in allergic states. It is possible that glycine or other amino acids, rather than the amino acids contained in a protein to which the patient is sensitive, can be utilized, and this may serve to correct the underlying cause of the disease. It is also quite possible that an allergic state might be due to a deficiency of a certain amino acid or group of these, due to a faulty digestion or absorption of protein. Since the amino acids themselves do not have to undergo any digestion in the intestine fairly large quantities of them can be ingested and the resulting increase in protein metabolism should be of much benefit to the patient.

CHAPTER XVIII

SIGNIFICANCE OF CREATINE CONTENT OF THE HEART. CHEMICAL NATURE OF HEART FAILURE

THE CREATINE content of the heart is about one-half that of skeletal muscle. The total P is about the same as that of skeletal muscle but a much greater part of it is present in the acid soluble fraction. This fraction is accordingly less and this difference may be traced to the lowered creatine phosphate content of the heart. The determination of creatine phosphate and adenylypyrophosphate is very difficult since these are very labile substances. Creatine, on the other hand, is easily determined and is usually taken as a measure of the creatine phosphate content of the heart.

Flossner (1) and du Vigneaud and Behrens (5) observed some interesting circulatory effects, including a lowering of blood pressure and bradycardia, when aqueous muscle extracts were injected to intact animals. Pace and Main (1) observed that a commercial meat extract diluted with K-free Ringer solution at the proper pH, had a marked inotropic effect upon the isolated perfused frog heart and upon guinea pig atrium. The active substance is soluble in water and 70 per cent alcohol, insoluble in ether, is stable at 95° C. and is destroyed by autoclaving in acid solution. Of all the substances tested creatine resembled the active substance in its

chemical properties. In concentrations from 0.0008 to 0.1 per cent, creatine, in 7 out of 10 experiments, caused increases in the amplitude of the heart beat from 20 to 50 per cent. Creatinine had no such effect. Backman (1) also found a positive inotropic effect of creatine on isolated rabbit heart and Shapiro (4) demonstrated a similar action with creatine and creatinine on perfused toad, rabbit, and cat hearts.

The important relations between creatine, phosphate and sugar metabolism in the heart and skeletal muscle are being actively investigated. During recent years the problem of myocardial failure has been attacked from the chemical point of view. The newer developments in the physiology of muscular contraction are being applied to the heart with future possibilities in the treatment of its various diseases.

Myers and Mangun (7) have published values for the normal and failing heart (see Table 47).

It should be observed that most of these so-called "normal" hearts represented hearts from individuals suffering from various diseases exclusive of heart disease. It might, therefore, be questionable, if the creatine content of these hearts can be accepted as representing the creatine content of the normal heart. Some of the analyses of Mangun and Myers (8), Herrmann and Dechard (2), and Constabel (1), however, were obtained from individuals killed by accident, and these hearts may be considered essentially normal. Linegar, *et al.* (4) stated that the normal creatine content of the left ventricle was 200 mg., and that of the right, 150 mg./100 gm. Bodansky, *et al.* (11) also showed that the creatine concentration was lower in the left than in the right ventricle in the newborn infant. His results in mg. per 100 gm. of heart were as follows: 5-6 month fetus, L.V. 45, R.V. 66; newborn female, L.V. 108, R.V. 131; newborn male, L.V. 78, R.V. 112. Seecof, *et al.* (12) on the other hand found that the creatine content of newborn infants was approximately

TABLE 47

THE CHEMICAL COMPOSITION OF THE HUMAN HEART
(After Mangun and Myers (7))

	No. of Cases	Left Vent. Mg./100 gm.	Right Vent. Mg./100 gm.	Reference
Water, Gm.				
Normal	5	78.9	79.2	Wilkins and Cullen (1)
Insufficiency	8	80.5	81.5	Wilkins and Cullen (1)
Chloride, Miscellaneous	10	139	182	Muntwyler, <i>et al.</i> (11)
Sodium,				
Normal	5	92	107	Wilkins and Cullen (1)
Insufficiency	8	115	142	Wilkins and Cullen (1)
Potassium,				
Normal	13	285	219	Mangun and Myers (8)
Normal	5	311	255	Wilkins and Cullen (1)
Insufficiency	8	258	200	Wilkins and Cullen (1)
Insufficiency	17	232	178	Mangun, <i>et al.</i> (9)
Total Phosphorus,				
Normal	13	194	160	Mangun and Myers (8)
Normal	5	203	177	Wilkins and Cullen (1)
Insufficiency	8	170	149	Wilkins and Cullen (1)
Insufficiency	17	169	135	Mangun, <i>et al.</i> (9)
Acid-Sol Phosphorus,				
Normal	36	89	...	Dechard and Blum (5)
Insufficiency	33	72	...	Dechard and Blum (5)
Creatine,				
Normal		221	173	Vollmer (1)
Normal	3	220-285	...	Bodansky and Bodansky (12)
Normal	48	...	117	Cowan (2)
Normal	95	208	149	Linegar, <i>et al.</i> (4)
Normal	34	175	...	Herrmann, <i>et al.</i> (2)
Normal		199	148	Mangun, <i>et al.</i> (9)
Normal	84	211	148	Seecof, <i>et al.</i> (12)
Normal (trauma)	13	203	165	Mangun and Myers (8)
Normal (trauma)	11	183	...	Herrmann, <i>et al.</i> (2)
Normal	48	202	...	Cowan (2)
Normal (M)	51	165	116	Bodansky, <i>et al.</i> (11)
Normal (M)	40	175	128	Bodansky, <i>et al.</i> (11)
Normal (F)	27	168	113	Bodansky, <i>et al.</i> (11)
Normal (F)	15	171	124	Bodansky, <i>et al.</i> (11)
Insufficiency	48	202	...	Cowan (2)
Insufficiency	11	175	132	Linegar, <i>et al.</i> (4)
Insufficiency	32	122	...	Herrmann, <i>et al.</i> (2)
Oxypurine Nitrogen,				
Miscellaneous	18	36.9	22.5	Mangun and Myers (10)
Insufficiency	6	30.0	22.4	Mangun and Myers (10)

the same in both ventricles. The maximum is reached in each ventricle at about 30 years of age.

Myers and Mangun (7) correlated heart weights with their creatine content. The creatine content of both ventricles is low in hearts weighing 50 to 150 gm. In the 150-

250 gm. hearts the right ventricle maintains its highest level up to 350 gm. of heart weight. It then begins to fall, reaching its lowest level in the 650 gm. hearts. It should be mentioned that age and myocardial insufficiency affect the creatine content of the heart, but even when these facts are taken into account there is still a statistical relationship between heart weight and its creatine content.

According to Myers and Mangun (7) the heart tends to maintain a value of 170 mg. of creatine per 100 gm. in the healthy individual. Mangun and Myers (4) found the heart creatine elevated in fevers and in uremia. They stated, "By the retention of creatinine, the creatine-creatinine equilibrium would be shifted toward the former." Mangun, *et al.* (9) also noted a tendency toward an elevation of potassium and phosphorus, suggesting the possibility that the extra creatine may be retained as the dipotassium salt of creatine phosphoric acid.

Linegar, *et al.* (4) determined the creatine content of 33 dog hearts. The average values for creatine were: left ventricle, 314 mg./100 gm.; right ventricle, 291 mg./100 gm., or a difference of 23 mg. or 7.3 per cent. It was also shown that creatine can be washed out of cardiac muscle by perfusion, which indicates that some of the creatine is present in the heart in diffusible form. Since the bound form of creatine is non-diffusible the creatine that does diffuse must be that obtained from the hydrolysis of creatine phosphate in the heart.

Linegar, *et al.* (4) have determined the creatine content of the left and right ventricles of the hearts of a number of patients suffering from different diseases (Table 48).

Thus it is seen that the concentrations of creatine in cardiac muscle are increased or normal in lobar pneumonia and uremia and *reduced* in heart failure. The creatine content of the heart is also lowered in 50 per cent of the cases of tuber-

culosis, in 20 per cent of the cases of acute infection, in 83 per cent of carcinoma, and in 75 per cent of diabetes. Likewise the creatine content of voluntary muscle is increased or normal in uremia and pneumonia, whereas it is lowered in all cases of diabetes and carcinoma, in 50 per cent of the cases of acute infection and in 60 per cent of cases of tuberculosis.

TABLE 48

SUMMARY OF AVERAGE DATA ON CREATINE CONTENT
OF CARDIAC AND PECTORALIS MAJOR MUSCLES
(After Linegar, *et al.* (4))

No. of Cases	CREATINE CONTENT				Grouping
	Left Vent., mg.	Right Vent., mg.	Diff., per cent	Pectoralis Major, mg.	
5	287	210	26.8	501	Uremia
5	237	153	35.4	441	Lobar pneumonia
15	223	151	32.3	393	Acute infections
20	208	148	28.8	412	Miscellaneous
9	206	159	23.3	328	Young human beings
14	206	158	28.2	382	Chronic infections
4	195	143	26.7	313	Diabetes
6	180	129	35.3	340	Carcinoma
6	176	136	22.3	401	Uremia with heart failure
11	175	132	24.6	390	Cardiac decompensation
95 (Average)	208	149	28.4	395	

Seecof, *et al.* (12) have made a very interesting study of the chemical differences between the left and right ventricles of the heart in several species of animals and in man. It was established that the concentration of creatine in the left ventricle is higher than in the right ventricle. When the creatine content exceeds 400 mg. (the probable saturation point) a corresponding increase in the cardiac muscle also occurs. It was also observed that the retention of creatinine in renal disease has an augmentating influence on the creatine content of both cardiac and voluntary muscle (*Cf.* Chapter IX on the transformation of creatinine into creatine). In addition to embryonic, anatomic, physiologic and pathologic observa-

tions, chemical evidence is presented showing that the left and right ventricles are different muscles (Table 49).

In their series of 85 human hearts the content of creatine in the left ventricle averaged 30 per cent higher than that in the right ventricle. This difference was 14 per cent in cattle

TABLE 49

CREATINE CONTENTS OF HUMAN HEARTS
(After Seecof, *et al.* (12))

No. of Cases	CREATINE—		Creatine Content of Heart, Mg./100 gm.	Grouping	Reference
	Left Vent., Mg./100 gm.	Right Vent., Mg./100 gm.			
11	175	132	...	Cardiac decompensation	Linegar, <i>et al.</i> Bodansky, <i>et</i> (4)
6	176	136	...	Uremia with heart failure	Linegar, <i>et al.</i> (4)
5	287	210	...	Uremia	Linegar, <i>et al.</i> (4)
50M	139	103	...	Congestive heart failure	Bodansky, <i>et</i> <i>al.</i> (11)
20F	146	108	...	Congestive heart failure	Bodansky, <i>et</i> <i>al.</i> (11)
39M	146	109	...	Cardiac hypertrophy	Bodansky, <i>et</i> <i>al.</i> (11)
16F	167	111	...	Cardiac hypertrophy	Bodansky, <i>et</i> <i>al.</i> (11)
22	175	Infectious diseases	Herrmann, <i>et</i> <i>al.</i> (2)
6	198	Hypertension without heart failure	Herrmann, <i>et</i> <i>al.</i> (2)
11	157	Coronary sclerosis without heart failure	Herrmann, <i>et</i> <i>al.</i> (2)
5	157	Severe anemia	Herrmann, <i>et</i> <i>al.</i> (2)
6	159	Glomerular nephritis with uremia	Herrmann, <i>et</i> <i>al.</i> (2)
4	...	Good Infarcted	151 46	Coronary thrombosis	Herrmann, <i>et</i> <i>al.</i> (2)
32	122	Congestive heart failure	Herrmann, <i>et</i> <i>al.</i> (2)
11	183	Trauma	Constabel (1)
33	172	Infectious diseases	Constabel (1)
34	175	Controls	Constabel (1)
6	198	Hypertension	Constabel (1)
11	157	Coronary sclerosis	Constabel (1)
18	173	Total without failure	Constabel (1)
6	159	Glomerular nephritis with uremia	Constabel (1)
5	157	Severe anemia	Constabel (1)
32	122	Congestive heart failure	Constabel (1)
10	121	Infectious diseases	Constabel (1)

hearts. Vollmer (1) found the left ventricle to be 21 per cent higher in creatine. This investigator also showed differences in glycogen, lipids, phosphoric acid, and inorganic salts, such as potassium and calcium. Katz (1) had already established that there was an asynchronism between the two ventricles, and that this could not be due to differences in pressure, tension and volume in the ventricular chambers, but that some unknown factors were involved. Seecof, *et al.* (12) believe that the asynchronism and electrocardiographic findings are due to the fact that the ventricles are qualitatively two different muscles (*Vide infra*).

It is of much interest to point out here that it could be predicted, from the known relation of creatine phosphate to the contraction process, that, since the left ventricle has the higher creatine content, its contractions should be faster, more energetic, etc., than the right, and Katz's findings confirm this prediction.

In the light of these important findings Seecof, *et al.* (12) stated that any factor which altered the creatine content of the heart may alter its function. This means that the heart, in one or other of its chambers, may be affected by different increases or decreases of creatine in various diseases. They also stated that these findings might have some value in therapeutic measures applied to the heart and suggested the ingestion of creatine precursors, *e.g.*, glycine, in cases of heart failure. Some of the increase in work output of the heart noticed by Freedburg, *et al.* (1) after administration of theobromine sodium acetate may have been due to the fact that the purines, in addition to their diuretic effects, are known to increase the creatine content of both skeletal and cardiac muscle. Hence the administration of the amino acids, purines or nucleotides in heart disease is based upon sound experimental facts.

The chemical nature of heart failure. Cardiac activity de-

depends on an adequate supply of oxygen, glycogen, creatine phosphate, adenylypyrophosphate and other components that may participate in the energy transformations of the myocardium. The heart is very sensitive to lack of oxygen and it has a small ability, as compared to that of skeletal muscle, of contracting an oxygen debt. Also the tendency for glycogen to increase, and of lactic acid to decrease, which accompanies anoxemia from any cause, has been established by several investigators (Katz and Long (2); Hines, Katz and Long (3); Katz, Kerrige and Long (4); Himwich, Goldforb and Nahum (1); and Meekins (1)). This leaves little doubt but that the chemical approach to the nature of heart failure is an important one and this aspect of the question has not received the attention that it deserves in the past. This was due to the fact that, before the discovery of creatine phosphate, no function of the creatine content of the heart was known. In recent years, however, this situation has greatly changed by the results of many recent investigators.

The fundamental question is one of the relation of heart failure to the creatine reserve of the heart. Bodansky (4), Bodansky and Duff (5, 9) and Bodansky, *et al.* (8) have shown that heart failure in the hyperthyroid rat is associated with a marked reduction of the creatine content of the myocardium. From an initial value of about 190 mg., which closely approximates the normal concentration in man, the creatine concentration may be reduced to 90 mg. Below this level circulatory failure usually supervenes when the creatine reserve has been reduced to about 50 per cent of normal. These results were obtained with over 300 rats treated with large doses of thyroid or thyroxin. In this condition it seems that the glycogen and creatine phosphate mechanisms are primarily affected and the loss in creatine follows that of creatine phosphate. It appears, however, that such factors

as may be expected to increase the load on either ventricle, thereby leading to exhaustion, are often associated with a lowered creatine reserve of the corresponding portion of the myocardium.

Since the first investigation on the creatine content of the human heart by Constabel (1) it has become apparent that this substance is *decreased* in the failing heart. On this point all investigators are agreed (Vollmer (1); Cowan (2); Linegar, *et al.* (4); Bodansky, *et al.* (11); and Herrmann, *et al.* (2, 3)). Analyses of the creatine content of the heart in cases of heart failure have been published by Constabel, Herrmann, Bodansky, Myers, and their co-workers. There is no change in the creatine content of the heart within 36 hours after death (106 cases reported by Seecof, *et al.* (12)). These average values are listed above in Table 49.

According to Herrmann and Dechard (2) heart failure in its simplest and commonest forms represents an inadequacy in contraction of a critical number of individual heart muscle cells. They stated, "Every clinician has witnessed the death of patients with evidences of complete myocardial insufficiency only to have pathologists offer nothing more than slight microscopic myocardial changes to account for the clinical picture. In such instances, we have hesitated to admit the histological abnormalities as full explanation of the complete insufficiency in myocardial function. On the other hand we have seen serious and extensive pathological processes demonstrated in the hearts of patients who during life have shown no evidence of primary heart failure. It has, therefore, been widely appreciated that anatomical findings in themselves often fail to show satisfactory correlation with the functional status of the organ."

Within recent years considerable work has been done on elucidating the cellular metabolism and biochemistry of the heart. It has long been suspected that derangement of the

physicochemical processes may occur in heart failure. Students of cardiac disease at the present time believe that the "failure" must be chemical rather than mechanical. In a series of 12 dogs Herrmann and Dechard (4) produced myocardial infarction by catheterization of the coronaries through the carotids followed by the injection of varying sized globules of mercury. Creatinuria resulted after two or three days and lasted about a week. The circulatory changes brought about in these experiments evidently contributed to the production of the creatinuria. Chemical analyses of the infarcted and normal muscle immediately following the infarction showed a marked drop in glycogen in the infarcted muscle, evidently the result of anoxemia. Edema in the infarcted area began to appear within half an hour. Analyses of the infarcted human heart also showed low creatine values, about one third that of the other heart muscles. Beltrametti (1) stated that during cardiac decompensation there is a marked increase in blood and urine creatine. This creatinuria disappears when the blood reaches equilibrium. The creatinuria of cardiac disease is due to a different cause from that of hyperthyroidism.

Burns and Cruickshank (1) studied the effects of asphyxia and fatigue on the excised heart of the cat. When the heart was stopped in certain stages of asphyxia a loss of creatine phosphate preceded that of adenylypyrophosphate. With complete asphyxiation there was a loss of 80 per cent of creatine phosphate and 60 per cent of adenylypyrophosphate. Similar results were obtained with the dog's heart. In the presence of oxygen, however, fatigue produced a 25 per cent loss in creatine phosphate and a 50 per cent loss in adenylypyrophosphate.

The central position occupied by adenylypyrophosphate in relation to creatine phosphate, glycogen, energy transfer and phosphorus transfer warrants further study. Mangun and

Myers (10) observed that there was a drop of about 15 per cent in the extractive oxypurine content in 6 cases of myocardial deficiency. There was also a drop in total acid soluble phosphorus and creatine in this series of hearts. In the group of hearts in insufficiency the total acid soluble phosphorus was about 15 per cent below that of the other cases while the creatine was 33 per cent lower. Burns and Cruickshank (1) stated that adenylypyrophosphate is greatly lowered in anoxemia and fatigue.

These observations also suggest that disturbances in the metabolism of adenylypyrophosphate may be of considerable significance in diseases of the heart. In Lohmann's reaction, $2 \text{ creatine} + \text{adenylypyrophosphate} \rightarrow 2 \text{ creatine phosphate} + \text{adenylic acid}$, creatine phosphate and adenylypyrophosphate exist in muscle in equilibrium with each other. Creatine phosphate acts as a donor of phosphate to keep the nucleotide in a full state of phosphorylation when the transfer of phosphate from phosphopyruvic acid is inadequate. In the failing heart this mechanism may be inadequate due to the depletion of the two compounds. Also the free adenylic acid would be deaminized into inosinic acid or hypoxanthine and ammonia and these compounds would be removed from the system until reamination could occur. It would be of great interest to learn more about the mechanism of reamination of inosinic acid to adenylic acid and of the effect of ingestion of amino acids and other compounds upon the process.

Herrmann and Dechard (2) observed in a series of over 500 adult human hearts that there were 374 from patients who died without showing definite evidence of congestive heart failure and 127 from patients who died in congestive heart failure. The hearts from the 374 patients who had not presented evidence of myocardial insufficiency showed average total creatinine levels of 175.3 ± 12.5 mg. per cent (standard deviation). In contrast the 127 hearts that had

failed contained an average of 125.5 ± 25.4 mg. per cent total creatinine. Table 50 from Herrmann and Dechard (2) contains the phosphorus values from these hearts. From this table it is also seen that the total phosphorus and acid soluble phosphorus are lowered significantly in the patients dying of congestive heart failure.

TABLE 50

PHOSPHORUS VALUES IN HUMAN HEART MUSCLE
(After Herrmann and Dechard (2))

<i>Cause of Death</i>	<i>No. of Cases</i>	PHOSPHORUS			
		<i>P, tot, mg. per cent</i>	<i>P, as, mg. per cent</i>	<i>P, lip, mg. per cent</i>	<i>P, o, mg. per cent</i>
Other than congestive heart failure	384	189	90.1	90.0	43.9
Congestive heart failure	127	162	72.4	89.5	41.8

P, tot, total phosphorus; P, as, acid soluble phosphorus; P, lip, lipid residual phosphorus; P, o, inorganic or other phosphorus.

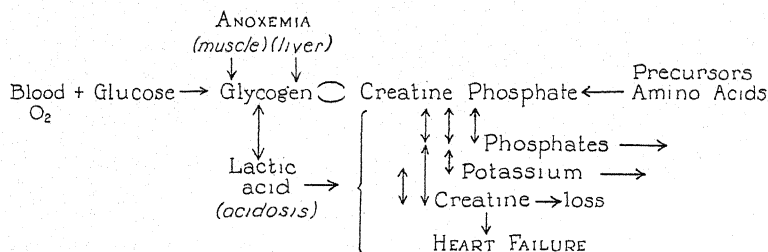
Herrmann and Dechard (2) conclude their interesting paper as follows: "In the light of these chemical findings it seems suggestive at least that total creatinine and creatine, total phosphorus and acid soluble phosphorus losses usually accompany heart failure. Whether the chemical changes are the cause or result of the heart failure is a question that should be raised and as yet cannot be answered. Further experimental evidence is needed. We must substantiate our contention that losses of total creatinine and phosphates are the factors that lead to myocardial weakness. It has been generally accepted that in anoxemia, asphyxia, and in aglycemia, there is a failure of resynthesis of creatine phosphate. These same conditions, we concluded, might lead to creatine and phosphate losses from human hearts and ultimate cardiac weakness. Consideration of such facts and our findings lead us to extend Harrison's (1) physical theory of anoxemic

etiology of circulatory failure to include inevitable biochemical changes in heart muscle to account for myocardial insufficiency."

Herrmann (5) draws up the schema shown in Fig. 6 to illustrate the chemical changes which occur in myocardial failure.

Fig. 6.

Schema of MYOCARDIAL FAILURE in terms of
PHYSIOLOGICAL CHEMISTRY ~



(A representation of present theoretical conception of the fundamental heart muscle cell biochemical changes responsible for heart failure, after Herrmann (5)).

(Courtesy of Doctor Herrmann and the C. V. Mosby Co., St. Louis, Mo.)

According to this schema the anoxemia disturbs the biochemical reaction of glycogen, creatine phosphate, creatine and lactic acid metabolism, which are very closely associated with muscular contraction, tone and efficiency. It is assumed that creatine phosphate breakdown functions in heart muscle as it does in skeletal muscle in supplying energy for contraction. Its resynthesis is indispensable to continued action. The inability of the anoxic skeletal muscle and liver to resynthesize lactic acid to glycogen causes this acid to accumulate in the blood stream with resulting acidosis. The anoxemia

further causes a loss of potassium and creatine, which is shown by analysis of mammalian and human hearts that have failed. This tends to produce a vicious cycle which would explain the progressive nature of heart failure. The loss of buffering base from the tissue results in the inability to acquire the normally large oxygen debt. This causes an increased permeability of the capillary wall and edema results. Herrmann states (5): "The precursors of creatine (*i.e.*, glycine), creatine phosphate, or its potassium salt, and glycogen, or methods of combating anoxemia may some day be found that will stay the progress of the failing heart."

The ingestion of amino acids, *e.g.*, glycine, should therefore prove of benefit in different types of heart disease. Since glycine has been most extensively used in the myopathies several clinicians have used it in the treatment of different cardiac conditions. Manca (1) observed that the ingestion of glycine improves the nutritive condition of the myocardium and the metabolism of creatine and creatinine with subsequent clinical improvement in patients with heart disease. Kalter (1) reported many cases of heart disease which have shown good results following treatment with glycine alone or combined with a liver preparation (glykhepar), sex hormones or adenosine triphosphate. The dose of glycine was about 15 gm. per day and two weeks sufficed for beneficial results to be observed. Seilor (1) likewise studied 50 cases of severe cardiac decompensation. Glycine and phosphates are valuable adjuncts to strophanthin therapy. The tolerance to this drug is increased 30 per cent by the glycine and phosphate preparation. Herrmann (1) stated that in several cases of what appeared to be severe infarction the outcome was favorable after glycine ingestion and that this form of medication deserved further trial. Further experimental and clinical studies of the failing heart with respect to changes in its different chemical constituents, and of the effect of glycine

and vitamin B₁ ingestion, will be awaited with much interest.

It should be remembered that the writer has shown that some of the purines and their derivatives which are used in the treatment of heart disease (*i.e.*, aminophyllin) also increase the formation and excretion of creatine and this may account for some of their therapeutic action in these cases.

Myers (14) has recently summarized the experimental work in the above field including his own. He now states that there is no difference in the creatine, phosphorus and potassium content of the two ventricles due to the fact that the low values found for the right ventricle were due to increased fat and connective tissue of the right as compared to the left ventricle. In the very early stages of cardiac hypertrophy there is a slight increase in creatine, phosphorus and potassium, but later a decrease in these constituents occurs and reaches their lowest values in extreme cardiac hypertrophy and heart failure. Myers believes, with Herrmann, that these chemical changes are the cause of heart failure.

This discussion would not be complete without mention of the important relation of vitamin B₁ to the chemistry of the heart. The "beriberi" heart and the effect of vitamin B₁ upon it is well known. In recent years, however, it has been clearly recognized by clinicians that myocardial dysfunction may follow deficiency of this vitamin. Eustis (1) reported the dramatic recovery of 2 cases of this syndrome after the administration of 10 to 60 mg. of thiamin chloride daily. Doctor Tripoli, in commenting on these cases, stated, "Dr. Eustis called our attention to one of the most important recent advances in the therapy of heart disease. In the past we believed that by definition a patient must present demonstrable polyneuritis, before a diagnosis of beriberi could be substantiated. We know now that syndromes of so-called subclinical beriberi may be manifest in the nervous system, cardiovascular system or gastrointestinal tract without the

complete picture being present. The dramatic result obtained by oral or parenteral administration of thiamin chloride in beriberi is incredible unless one actually sees it."

Since vitamin B₁ is so closely connected with carbohydrate metabolism, and this type of metabolism is in turn very closely related to creatine metabolism, and since we and others have shown that vitamin B₁ administration to normal males serves to increase their energy output, it is clearly seen that the metabolic relation of creatine, carbohydrate and vitamin B₁ is a very important one, and the author predicts that the clinicians in the future should take these facts into consideration in regard to the etiology and treatment of some diseases of the heart.

CHAPTER XIX

EXCRETION OF CREATINE AND CREATININE IN VARIOUS CLINICAL CONDITIONS

AS STATED in Chapter VIII creatine excretion is by no means specific for diseases of the muscles. Creatinuria has been observed in diseases of the heart, in hyperthyroidism, myxedema, in normal and hyperthyroid children, in sclerodermia, fevers, dinitrophenol ingestion, phlebitis profunda, after fractures, in malignant disease, diseases of the liver, toxemias of pregnancy, in tetany following parathyroidectomy, after thyroid administration, changes of external temperature, etc.

E. Wang (1) reviewed the subject of creatinuria. According to Wang, "One of the puzzling features of creatine metabolism is the great diversity of conditions known to cause creatinuria. The consideration of this point is given a much broader significance by the assumption that creatinuria is due to an action upon creatine phosphate which is a compound of very intimate relation to other substances. It is a link in a system of several cooperating constituents, of which may be mentioned adenylypyrophosphate on the one hand and a series of carbohydrate disintegration products on the other. It may be assumed that a disturbance affecting any point of this system will influence all of the compounds con-

cerned. Accordingly, the various conditions causing creatinuria must not necessarily be supposed to act upon the creatine phosphate itself, but may as well be attacking any other link in the chain."

Wang's experimental work was done on urine, blood, and muscles both in patients and animals. On the usual hospital food it was found that the average daily creatine excretion for men was 18 mg. and for women 48 mg., although the maximum amounts were about 4 times as large. The creatine excretion in thyreotoxocosis was independent of the basal metabolism, but seemed to be related to thyroxin itself. During treatment with iodine the creatinuria decreases parallel to the general improvement. At the same time the creatinine excretion diminishes somewhat in direct ratio to the severity of the thyreotoxocosis and during iodine treatment remains unaltered. Creatinuria is frequently associated with fevers, but does not seem to depend so much upon the severity of the febrile condition as upon the extent to which the patient is affected. The creatinine excretion in fever is below normal. As far as uremia, hypertension, cardiac insufficiency, etc., are concerned, the creatine and creatinine excretions are not affected. Phlebitis profunda is associated with a profound creatinuria which increases and decreases with the clinical condition. The creatinine excretion in these cases is low. Creatinuria is present in poliomyelitis and is proportional to the severity of the disease. During improvement the creatinuria recedes in a parallel manner with the improvement. The creatinuria serves as a measure of the severity of the disease. Generally in the early stages of the disease, the creatinine excretion decreases. After fractures, or sciatica, but not with arthritis associated with atrophy of the muscles, there is an increased excretion of creatine, but not in creatinine excretion. From these results Wang concluded that creatinuria is an entirely unspecific phenomenon, but is of clinical value

as an index of the condition of the muscles affected in various diseases.

Increased creatine excretion has been reported in 400 mentally defective patients (Penrose and Pugh (1)). Those suffering from muscular dystrophy showed a high creatine and low creatinine excretion. In children with diplegia these same variations were observed. In mongols and epileptics the creatine excretion was normal. There was a high creatinine excretion in cerebellar ataxia and postencephalitis lethargica. Stora and Tcherneakosky (1) observed hypercreatinuria in 14 out of 29 cases of dementia praecox. Cohen and Fierman (1) observed creatinuria in 8 male schizophrenics subjected to prolonged periods of large doses of dessicated thyroid. There was no change in the excretion of creatinine. Horvath and Corwin (4) also observed creatine excretion in schizophrenics and the creatinine excretion was not as constant as has been assumed in the past. Pugh (2) observed creatinuria in 4 patients with phenylketonuria which was probably due to hyperthyroidism. Creatinuria was reported in 2 cases of dermatomyositis by Milhorat, *et al.* (12) and in patients with chronic enteritis by Bohn and Basler (2).

The excretion of creatine and creatinine in 134 cases (63 males and 71 females, ages from 14 to 85 years) was studied. Nerve and conduction deafness was studied in eight (Selfridge and Beard, unpublished observations) before therapy was instituted. If 100 mg. of creatine excretion is considered physiological (due to the fact that women are known to excrete small amounts of creatine and to the fact that creatinine determinations with the photoelectric colorimeter are more accurate than with the eye) then 72 per cent of these cases showed a creatinuria ranging from 100 to 1,220 mg. in the 24-hour urine specimen. The correlation coefficient between age and creatine excretion was 0.21 ± 0.06 , showing that little, if any, relation was observed between these two

variables. Based on these observations it was concluded that a possible muscular dystrophy of the tensor, tympani and stapedus muscles was present. The dietary histories of these patients was studied in detail and indicate that environmental rather than hereditary factors are perhaps more important in conduction deafness. Chronic progressive deafness is related to disorders of nutrition and not to any one single factor, and in adhesive deafness the outstanding factor appears to be an unresorbed mesenchyme, due to a lack of the intracellular cementum substance—cevitamic acid. Treatment of these cases with glycine, nicotinic acid, vitamins B₁, C, etc., has yielded encouraging results.

Thorn and Tierney (4) studied a case of thyrotoxicosis and myasthenia gravis in the same patient. Improvement in muscle function following subtotal thyroidectomy was observed. The creatine excretion after iodine therapy fell from 420 mg. to about 50 mg. per day followed by improvement in the creatine tolerance test. Prostigmin was also administered and patient was so improved that she was able to resume her teaching duties.

Sutton (1) studied the relation of androgens to prostatic enlargement. Normal young males, after the administration of exogenous creatine, show a creatine retention above 70 per cent. Elderly non-prostatic individuals after the administration of creatine show a creatine retention comparable to that of normal young males. Elderly prostatic patients, after the administration of creatine, likewise show a creatine retention similar to that of young males and elderly non-prostatic individuals. Testosterone propionate administered to young and old males did not influence creatinine excretion. It appears that creatine retention after administration of creatine, is largely dependent on the androgenic substances formed by the adult testes, while in the absence of the testes there was a markedly impaired creatine retention.

CHAPTER XX

RELATION OF AMINO ACIDS, AMINES AND GUANIDINE BASES TO TUMOR GROWTH AND REGRESSION

FOR A number of years intensive study has been made by numerous workers to determine differences in the metabolic aspects of the cancer cell as compared to those of the normal cell. Soot, tar, carcinogenic hydrocarbons, viruses, irritation, endocrine imbalance, especially of the sex hormones, excessive sunlight or ultraviolet light, are all known to cause the change from normal into cancer cells (*Cf.* Beard (45)). While most interest at the present time is in the production of tumors by these agents some studies have also been conducted in which attempts were made to stop the growth of the tumors and cause their disappearance. Cancer cells have lost the power of differentiation and organization so characteristic of normal cells, but their tendency to proliferate is prodigious. One of our recent control rats bearing two Emge sarcoma transplants weighed 125 gm., while the weight of the large tumor mass removed from its body was 260 gm. It would seem, therefore, that any substance which can prevent this excessive proliferation and increase differentiation and organization of cells, should receive attention in experimental cancer research.

The first substances that come to mind in this connection are the amino acids, proteins, nucleoproteins, vitamins, enzymes and hormones. Since the cancer is a parasite it robs

the body of these essential constituents and utilizes them to promote its own growth. This creates a deficiency or imbalance of these substances with the result that the cancer tissue absorbs most of the constituents of the diet as well as of the animal's tissues, cachexia intervenes and the emaciated animal finally dies.

A number of years ago the writer became interested in studying the metabolic aspects of the cancer cell (Beard, *et al.* (46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58)). In our early studies a water extract of the suprarenal cortex of sheep and cattle was used after removing the proteins with alcohol. From 10 to 15 per cent of the Walker sarcomas disappeared in our rats injected with this extract (46, 47, 50, 51). Even before our experimental work was started this same extract had been shown to cause 50 out of 100 patients (from a total of over 7,500) suffering from hopeless malignancies to completely lose their tumors and live much longer than the usual 5 years which would have been allowed them by cancerologists (Coffey and Humber (1, 2)). Later several different amino acids were isolated from this extract (Beard (48)).

Several studies using all of the amino acids of the protein molecule, as well as the 10 isolated from the above extract, have recently been completed (56, 57, 58). In all of these studies young rats bearing double transplants of the Emge sarcoma were used. If the solution was injected the day of transplants it caused 83 per cent of the tumors to disappear and to slow the growth of those that remained on the animal (56). If two weeks were allowed to pass and the solution injected for the next 5 weeks, then only 9 per cent of the tumors disappeared (57). Large tumors sometimes disappeared, in one case one weighing 100 gm. disappeared but the strain finally killed the animal. There was only about 2 or 3 per cent of the untreated control tumors that disappeared. This work was then repeated using all of the amino acids

alone of the protein molecule, except tyrosine. In all 47 per cent of the tumors completely disappeared and the growth of many others was retarded (58). As a result of this work the amino acids were rated from highest to lowest on their ability to stop the growth of the tumors or to cause their disappearance, as follows:

Average Growth of Tumors: 1-leucine*, phenylalanine*; 2-proline, alanine, arginine + histidine*; 3-serine; 4-tryptophane*; 5-valine*, threonine*, glutamic acid, hydroxyproline; 6-cystine, isoleucine*; 7-aspartic acid; 8-methionine*; 9-norleucine; 10-lysine*; 11-cysteine, glycine.

Disappearance of Tumors: 1-arginine + histidine*; 2-arginine; 3-lysine*; 4-phenylalanine*; 5-valine*; 6-tryptophane*; 7-alanine; 8-methionine*; 9-histidine*; 10-leucine*; 11-norleucine; 12-isoleucine*; 13-aspartic acid; 14-proline; 15-glutamic acid; 16-glycine; 17-cysteine; 18-hydroxyproline; 20-threonine*.

Those amino acids with a star are known today as the essential amino acids, *i.e.*, those that cannot be synthesized by the animal and must be fed in the diet if nutritive disaster is to be averted. It is of much interest in this connection to observe that the diamino acid, arginine, was the most effective in causing the tumors to disappear, followed closely by several of the essential amino acids, while the non-essential amino acids, such as glutamic and aspartic acids, did not have much effect in this connection.

Hammett (2) has summarized his experimental work on the relation of the amino acids to the different growth phases of *Obelia*. In regard to arginine, aspartic and glutamic acids he stated, "It has been found that the developmental activity of differentiation is retarded by arginine and forwarded by glutamic and aspartic acids. Cytological studies indicate that the cancer cell does not differentiate to a normal degree. It does not mature as do other cells. The way is, therefore,

opened to see whether or not the immaturity of the cancer cell is associated with an imbalance between the forwarding amino acids and arginine. It is hardly necessary to point out all possible lines of attack which are here opened up. What the results will be in a practical way is for the future to tell. But that there are possibilities, no one will deny."

What is the relation of the above results to creatine metabolism? Since several workers, including ourselves, have shown that amino acids may inhibit and stop cancer growth and the evidence given in this monograph shows that creatine arises from the amino acids, it is, therefore, not unreasonable to expect that there should be some biological connection between creatine and malignant metabolism through the medium of the amino acids.

Let us now list some evidence for this view. Roffo (1, 2) stated that, since tumors seldom arise on muscular tissue, this tissue must contain substances that are detrimental to the origin and growth of tumors. This view is in line with the well known fact that different tissues contain factors which both accelerate and inhibit tumor growth (*Cf.* Boyland (2)). Roffo used a hydrolysate of muscle tissue and observed a dramatic disappearance of all tumors on his rats in three weeks. In a personal communication to the writer he stated: "The tumor regression obtained in these animals which gave me some hope at first did not take place in human tumors nor in rats with spontaneous malignancies. I think, therefore, that the muscle hydrolysate and its derivatives do influence the biological ground producing a biochemical modification. It is a satisfaction to me to learn that you also thought of the amino acids, which is probably in conformity with some of my experiments performed more than a year ago. Concerning this point I will gladly give you some details which you may add to your communication on the subject: I have isolated from muscle tissue amino acids, proteins and polypeptids. The

proteins and polypeptids tested in rats did not produce any difference in tumor growth, while all of the amino acids and other substances tested in these experiments (*d*-glutamic acid, leucine, *l*-histidine-HCl, glycyl-*l*-leucine, glycyl-*dl*-leucine, *dl*-alanyl-glycine, xanthine, hypoxanthine, guanine, and creatine) caused the complete disappearance of the tumors."

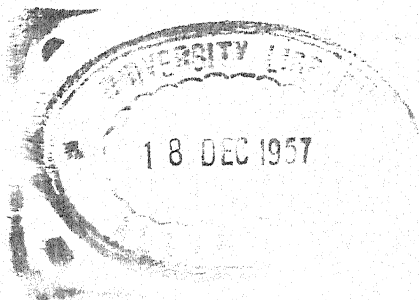
Amino acids have long been known to be inhibitory to growth of cells in tissue culture (Baker and Carrel (1) and Vogelaar and Erlichmann (1)).

Lustig and Wachtel (1) reported that guanidine acetic acid and creatine destroyed tumor cells *in vitro*, and that guanidine derivatives prolonged the life of tumor bearing animals. In another study they showed that lysine and guanidine would likewise cause complete tumor inhibition (2). Kline, *et al.* (1) showed that lysine and guanidine in some cases inhibited the takes of the Flexner-Jobling rat carcinoma, while β -indole proprionic and butyric acids were very effective in this connection. Lewisohn, *et al.* (1) observed that 30 per cent regressions of spontaneous adenocarcinoma could be obtained in the mouse by administering yeast extracts. The active material was precipitated with lead acetate, silver nitrate and phosphotungstic acids—properties showing the presence of nitrogenous bases or basic amino acids, such as arginine, histidine and lysine.

Boyland (2) showed that several of the amines isolated from heart muscle would inhibit the growth of grafted sarcomata and spontaneous carcinoma in mice. The per cent inhibitions were: cadaverine-HCl, 123; ethanolamine, 109; muscle extract, 50 to 115; trimethylamine-HCl, 87; phosphotungstic acid precipitate, 79; betaine, 42; carnosine nitrate, 77; choline-HCl, 75; creatine, 59; creatinine, 56. Brues, *et al.* (1, 2) have shown that most aliphatic amines are inhibitory to normal cell growth in tissue culture. Ethanolamine was the most active of all. The fact that this amine may take part in

the methylation of glycoxyamine to creatine was shown in Chapter VII.

From the above results we see that three different sets of workers have observed that creatine and its derivatives or precursors in the body, as well as creatinine in one instance, will cause tumors to disappear. It is, therefore, logical to believe that the above results may open up new lines of attack upon the cancer problem. To theorize as to the mechanism of the effect of the amino acids, bases, etc., upon cancer tissue would be unprofitable at the present time. From our own results it seems that the amino acids do not prevent cancer takes. On the other hand many tumors do not grow and many others completely disappear. Necrosis of the cancer cells occurs so that it is fairly certain that the amino acids are not able to change the cancer cell back into a normal one. It is possible that the writers' two lines of research (cancer and creatine metabolism) may merge in the future and we hope to be able to discover the proper combinations and amounts of the amino acids and other compounds that will consistently cause large numbers of growing tumors to disappear.





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